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Solid and solution phase combinatorial chemistry

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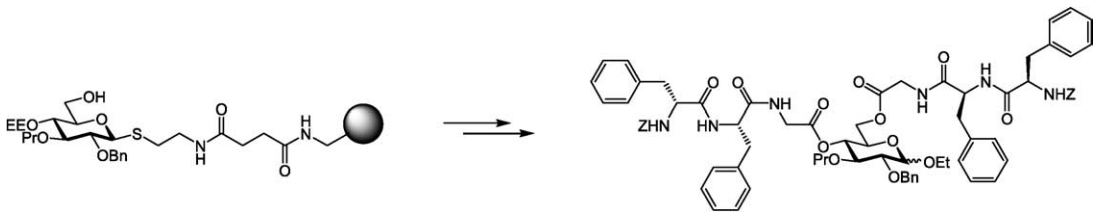
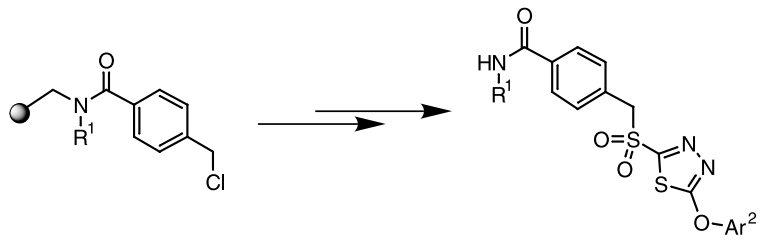
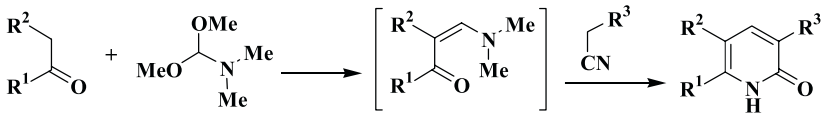
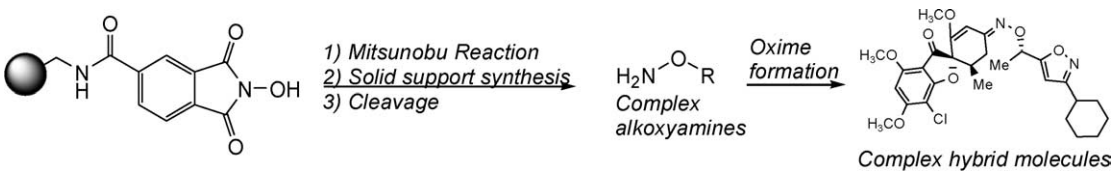
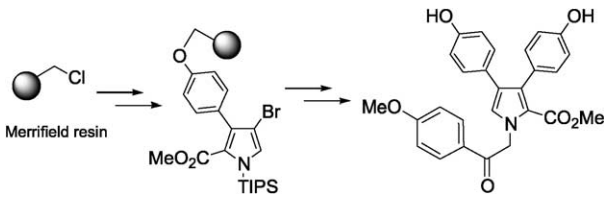


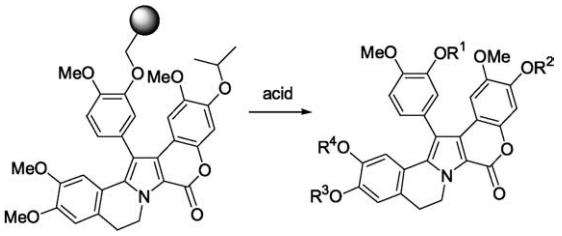


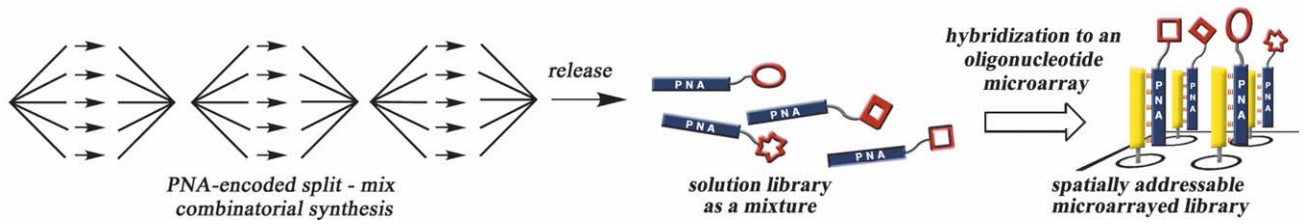


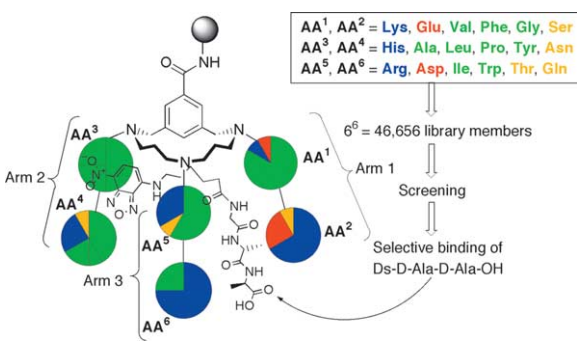


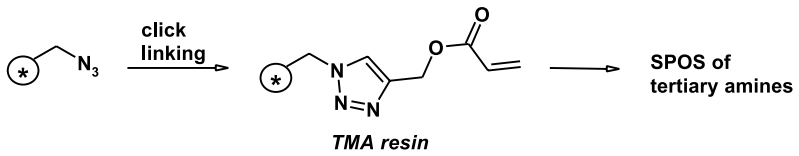


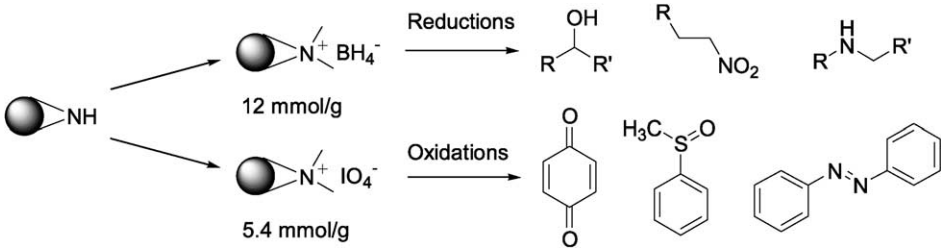
The solid-phase synthesis of lamellarins Q and O using Merrifield resin and a *N*-protected methyl 3,4-dibromopyrrole-2-carboxylate as a scaffold is described.

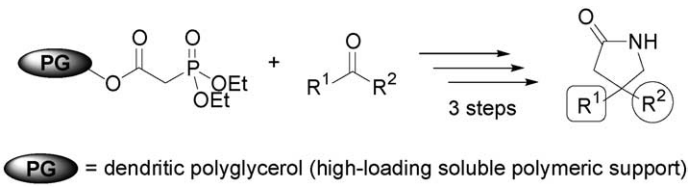


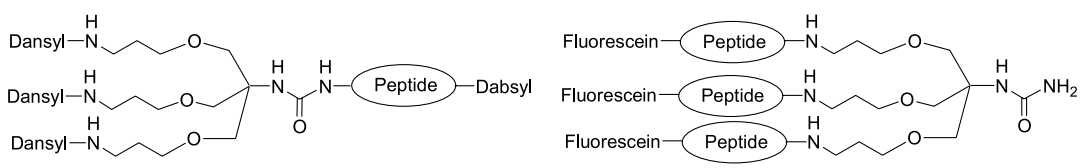


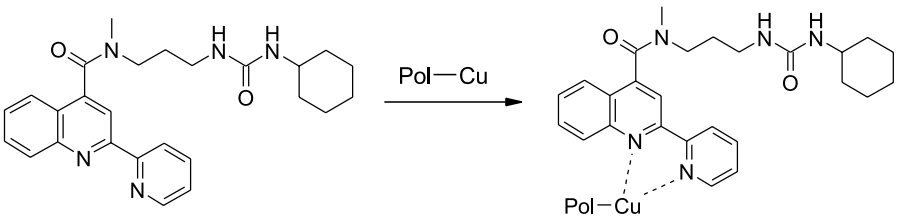












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Preface

During the last decade Combinatorial Chemistry and Solid and Solution Phase Synthesis of compound libraries have been established as the key technology for the preparation of compound collections with a predetermined set of properties. In particular, in medicinal chemistry and chemical biology research this synthesis technology had and will continue to have a major impact.

After more than a decade after the inauguration of the field it is appropriate to get an overview of the state-of-the-art and the 'Tetrahedron Symposium-in-Print' published in this issue is dedicated to this aim.[1]

From the various articles contributed by the different authors it is clearly visible that the field has matured. The kind of compound collections accessible today has become structurally much more challenging than in the early days of the field and the synthetic methodology required to prepare them now appears to be in full development. The breadth of the chemistry and its application to the various target classes reported in this issue support this conviction.

However, the development of compound library synthesis to a highly efficient routine technique has only just begun and we are convinced that the best is yet to come!

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Solid-phase synthesis of isoindolinones and naturally-occurring benzobutyrolactones (phthalides) using a cyclative-cleavage approach

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Abstract—Starting from Merrifield resin, 2-formylbenzoic acids were immobilized on solid supports. Reactions between immobilized 2-formylbenzoic acids and different organometallic reagents (Grignard reagents, zinc reagents, allyl silanes via Sakurai type reactions) furnished secondary alcohols which cyclized depending on the metal counter ion and reaction conditions, forming benzoannelated lactones. Asymmetric synthesis was possible on the resin using chiral [2.2]paracyclophane ligands. While the reaction of immobilized *ortho*-carboxy benzaldehydes with primary amines at elevated temperatures yielded 3-hydroxyisoindolinones, a reaction at ambient temperature allowed imine formation, which underwent 1,2-addition-cleavage reaction with various nucleophiles, yielding isoindolinones with three points of diversity.

1. Introduction

Natural products play a pivotal role in modern drug discovery. Therefore, the access to natural product libraries remains one of the foundations in this progress.[1,2]

Among the class of oxygen heterocycles, benzoannelated butyrolactones (phthalides) are present in various natural products.[3]

In particular, the 3-alkylated phthalides are present in some natural products such as Fuscinarin,[4] 3-butylphthalide, (−)-hydrastine ((−)-narcotine),[5] (−)-noscapine,[6] (−)-typhaphthalide,[7] spirolaxine,[8] (+)-monascodilone,[9] isoochracinic acid,[10] cryphonectric acid peracetate methyl ester,[11] vermistatin,[12] (−)-rubiginone-H,[13] alcyopterosin E,[14] and cytosporone E[15] (Fig. 1). Phthalides, such as the aforementioned, possess a wide range of biological activity. They are active at the opiod receptor ((−)-hydrastine), also known as the human CCR5 receptor, an important anti HIV-1 target, which interferes with HIV entry into cells (fuscinarin).[4] Some members of this group are cytotoxic (vermistatin, alcyopterosin E[14]) or antibacterial (e.g., cytosporone E[15] and related compounds[16]). 3-Butylphthalide, a constituent in the Chinese folk medicine extracted from celery seed oil,[17] reduces brain damage in mice.[18] It has been used for seasoning and flavoring purposes, shows anticonvulsant action,[19] increases the duration of anesthesia,[20] and exhibits cerebral antiischemic action.[21] Various naturally occurring phthalides, such as 3-butylphthalide from *Angelica sinensis* roots, or synthetic 3-alkenylphthalides showed muscle relaxant effects on animal tracheal smooth muscle, indicating that the phthalide moiety is the principal antiasthmatic component of phthalide derivatives of *Angelica* extractions.[22] In addition, the class of most of these chiral natural products are found only as one enantiomer. Because biological activity is strongly dependent on their configuration, synthesizing drugs and other biological compounds asymmetrically is highly desirable.

The analogous six-membered lactones are present in the very important class of ochratoxins (Fig. 2).[23]

Another class of potential biologically and pharmacologically active compounds are benzoannelated nitrogen heterocycles.[24] In this case, isoindolinones (phthalimidines) should be specifically mentioned.[25] taliscanine,[26] which occurs in the rhizomes of *Aristolochia taliscanina*, enterocarpam II,[27] isolated from the stem bark of *Orophea enterocarpa* (*Annoniacae*), or velutinam[28] from extracts of leaves and twigs of *Goniothalamus velutinus* belong to the aristolactams. Aristolactams are a minor group of

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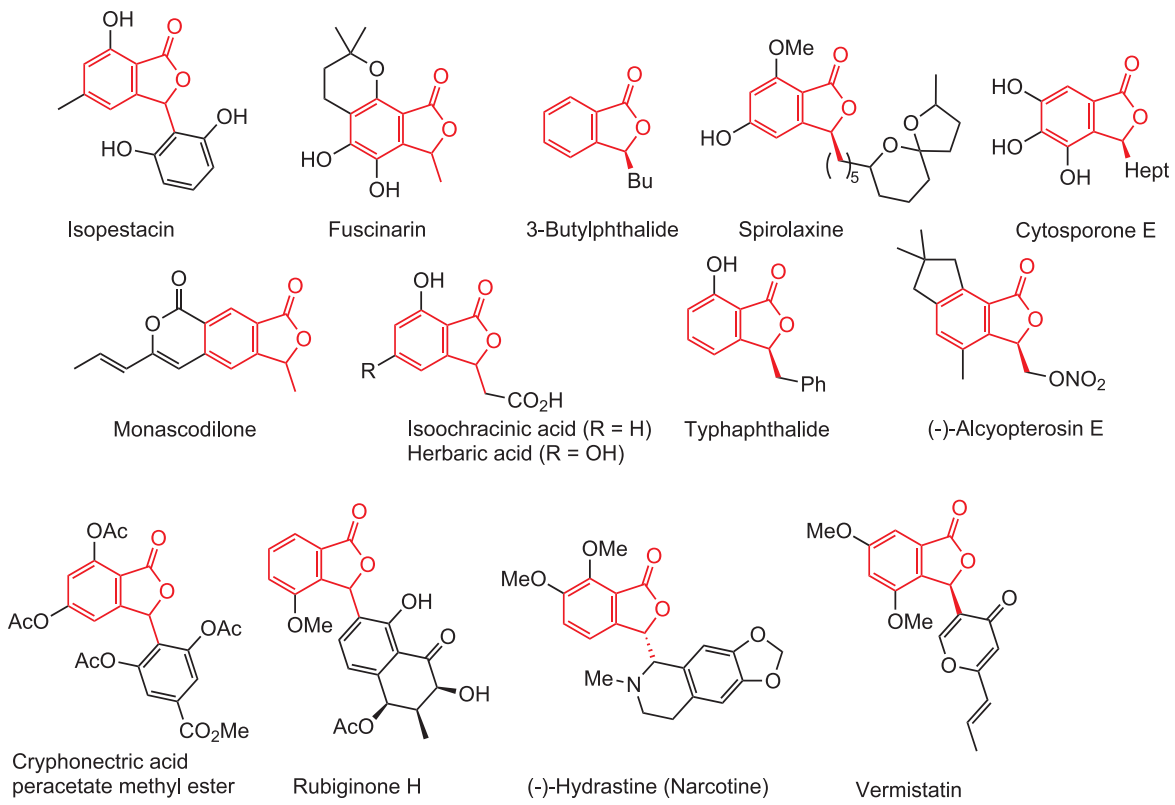


Figure 1. Some naturally occurring 3-alkyl and 3-aryl phthalides.

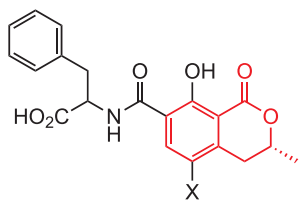


Figure 2. Structure of ochratoxins.

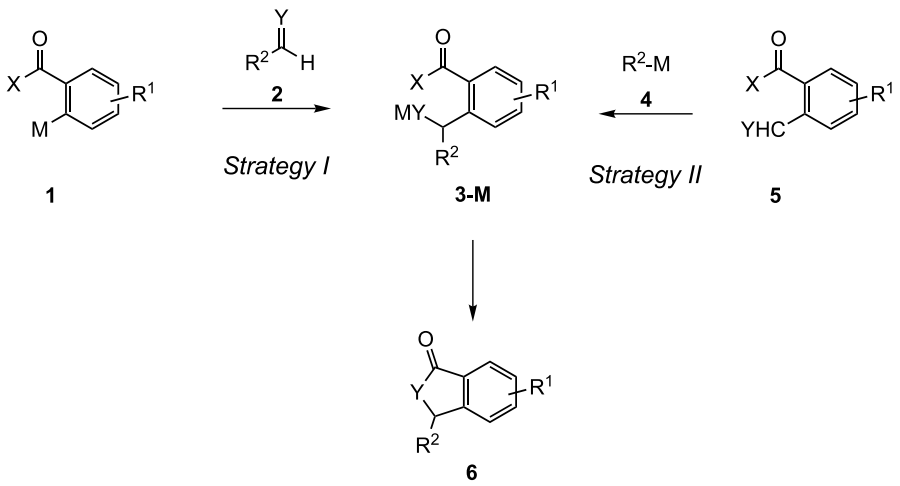
aporphinoid alkaloids biogenetically derived from isoquinolines. Phenanthrene lactams are used in folk medicine[29] as immunostimulant and anticancer agents.[30] The phenylidene derivative AKS 186 and its *O*-methylated analogue have been reported as inhibiting the thromboxane A2 analogue (U-46619)-induced vasoconstriction.[25a,b] Isoindolinone L-709,780 inhibits ADP-induced platelet aggregation with an IC_{50} of 27 nM.[31] In 1996, Egbertson et al. reported two- to three-fold improvement in potency over L-709,780 by introducing its sulfonamide derivatives.[32] Nofedone is an antiarrhythmic agent, it has been reported that in the treatment of ventricular arrhythmias, this drug was effective with quinidine-like action.[33] But one of the most famous representative compounds with a isoindolinone structure is thalidomide (Contergan®),[34] which is still used as a drug for the treatment of painful inflammations associated with leprosy,[35] rheumatoid arthritis,[36] and graft versus host disease (Fig. 3).[37]

For a modular synthesis of naturally occurring 3-substituted phthalides or isoindolinones, two strategies are applicable. The first originates with an *ortho* metallated ester **1** (X=OR), amide **2** ($X=NR_2$), or their synthon. Reaction with an aldehyde (**2**, Y=O) or an imine (**2**, Y=NR) and subsequent cyclization would lead to lactones **6** (Scheme 1, strategy I, Y=O) or isoindolinones **6** (Y=NR), respectively. This strategy has been used for the solid phase synthesis of phthalides.[38]

The other strategy is based on formyl-substituted benzoic acid derivatives **5** (Y=O) or their imines (Y=NR). Treatment with organometallic reagents such as alkyl lithium,[39] alkyl zinc,[40] alkyl sodium,[41] alkyl titanium,[42] or Grignard reagents[43] furnishes during or after acidic treatment the corresponding 3-substituted lactones (Scheme 1, strategy II, Y=O) or isoindolinones **6** (Y=NR), respectively. The second strategy is particularly interesting for an asymmetric variant, since the organometallic species can be purified before use (e.g., removal of salts) and can also be symmetric in the case of divalent metals (e.g., R_2Zn).

Since the required 2-formyl benzoic acids building blocks can be readily immobilized via an ester linkage, we decided to investigate this reaction on solid supports. Our primary intention was to examine the scope and limitation of this cyclization/cleavage approach with different nucleophiles. Simple starting materials were chosen as model compounds.

Therefore, commercially available 2-formyl benzoic acids **8** and **9** were immobilized on Merrifield resin **7** using standard conditions. To our knowledge, these resins have not been prepared before (Scheme 2).



Scheme 1. Strategies for the synthesis of 3-substituted phthalides (Y=O) and isoindolinones (Y=NR).

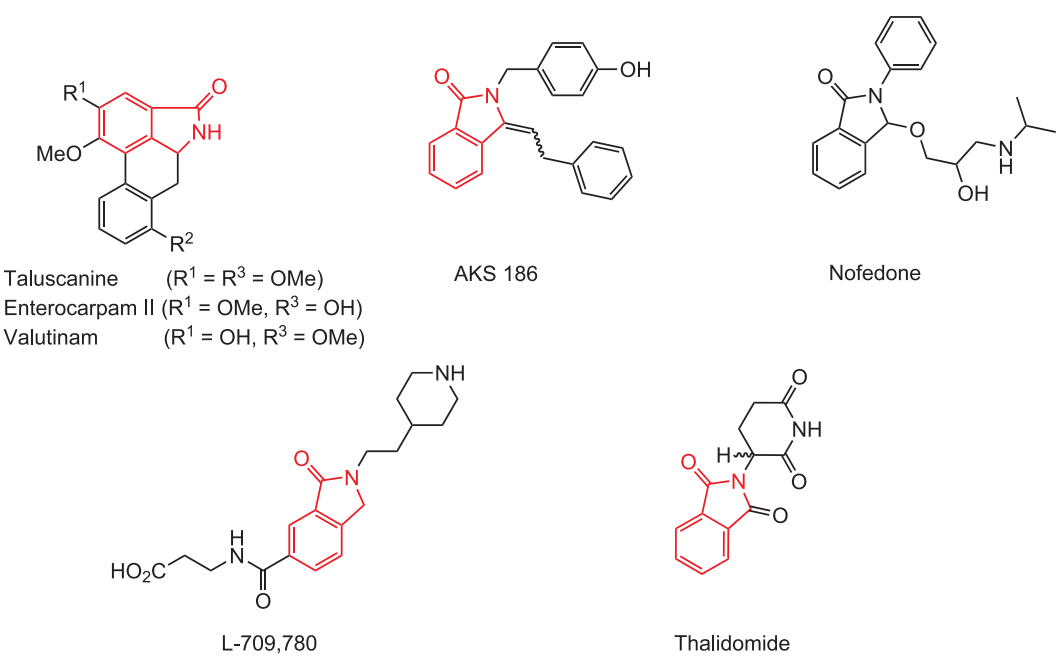
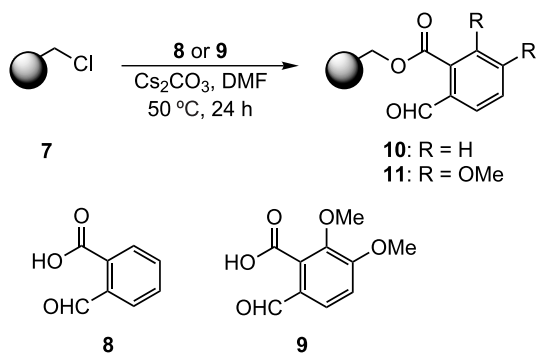


Figure 3. Natural products and biologically and pharmacologically active isoindolinones.

The resin **10** was then reacted with different organometallic reagents under various conditions (Tables 1–4). Reactions with lithium reagents (MeLi, EtLi, BuLi) led to cleavage at low temperature (−70 °C). However, an inseparable mixture of various alcohols was also isolated. In contrast, Grignard reagents react at low temperature (−70 °C) selectively to give the phthalides **13** in good purities, with the exception of ethyl magnesium chloride being not reactive enough for addition/cleavage at this temperature. It is important to note that the reaction at higher temperature with various Grignard reagents results in an addition reaction to the ester, which produces tertiary alcohols **15**. Reduction of the formyl group to a hydroxymethyl group to give benzyl alcohols **14** was also observed for ethyl magnesium chloride (Scheme 3).



Scheme 2. Immobilization of the benzoic acids **8** and **9** on Merrifield resin **7**.

Zinc reagents react at higher temperature in the presence of an amino alcohol such as *N*,*N*-dimethylamino ethanol selectively to give the phthalides **13b–d** in good purities and moderate overall yields. The formation of a reduction product or addition to the carbonyl group was not observed (Scheme 4).

Table 1. Addition of Grignard reagents to resin **10** (see Scheme 3)

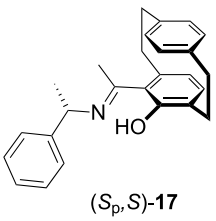
Grignard reagent	Solvent	T (°C)	t (h)	Product	R	Yield (%)[a]	Purity (%)[b]
MeMgCl	THF	−70	20	**13a**	Me	15	89
MeMgCl	THF	−10	20	**15a**	Me	36	n.d.
EtMgCl	THF	−70	20	—[c]	Et	—	—
EtMgCl	THF	−10	20	**14a/15a**	Et	17/11	n.d.
*i*PrMgCl	THF	−70	20	**13c**	*i*Pr	16	87
*i*PrMgCl	THF	−10	20	—[d]	*i*Pr	—	—
*n*BuMgCl	THF	−70	20	**13d**	*n*Bu	23	85
*n*BuMgCl	THF	−10	20	**15d**	*n*Bu	27	n.d.
PhMgCl	THF	−70	20	**13e**	Ph	13	85
(*E*)-$CH_3CH{=}CHMgCl$	THF	−70	20	**13f**[22,27]	$CH{=}CHCH_3$	15	74

[a] Overall isolated yields of purified compounds.
[b] Purity of the crude mixture after cleavage determined by GC.
[c] No conversion was observed.
[d] Inseparable mixture of products.

Table 2. Addition of zinc reagents to resin **10**

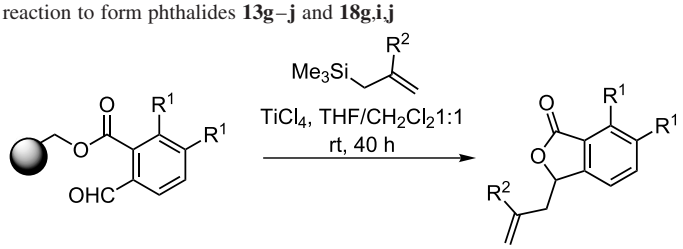
R_2Zn	Ligand	Product	Yield (%)[a]	Purity (%)[b]	ee (%)[c]
Me_2Zn	(S_p,*S*)-**17**	**13a**	—[d]	—	—
Et_2Zn	Dimethylamino ethanol	**13b**	14	80	0
Et_2Zn	(S_p,*S*)-**17**	**13b**	21	85	37
iPr_2Zn	(S_p,*S*)-**17**	**13c**	19	88	60
nBu_2Zn	Dimethylamino ethanol	**13d**	35	82	0
nBu_2Zn	(S_p,*S*)-**17**	**13d**	25	86	35

[a] Overall isolated yields of purified compounds.
[b] Purity of the crude mixture after cleavage determined by GC.
[c] Determined on a chiral stationary phase.
[d] No conversion was observed.



(S_p,*S*)-**17**

Table 3. Sakurai-type reaction to form phthalides **13g–j** and **18g,i,j**



10 (R^1 = H)
11 (R^1 = OMe)

13g-j (R^1 = H, R^2 = H, CH_2OCOCH_3, CH_2Cl, $SiMe_3$)
18g,i,j (R^1 = OMe, R^2 = H, CH_2Cl, $SiMe_3$)

Resin	R^1	R^2	Product	Yield (%)	Purity (%)
10	H	H	**13g**	73	95
10	H	CH_2OCOCH_3	**13h**	67	85
10	H	CH_2Cl	**13i**	69	90
10	H	$SiMe_3$	**13j**	51	86
11	OMe	H	**18g**	51	88
11	OMe	CH_2Cl	**18i**	24	76
11	OMe	$SiMe_3$	**18j**	60	75

At this point, it should be noted that it is important to exclude any air from the system due to the formation of alkoxides, which lead to acetals **16** in 19% yield.[44] However, the main advantage of using zinc reagents is, the possibility of stereoinduction by using chiral ligands. In 2001 and 2002, we introduced the [2.2]paracyclophane ketimine system as an effective ligand system for the asymmetric 1,2-addition of zinc reagents to aldehydes[45] and imines.[46] In the presence of ligand (S_p,*S*)-**17**, scalemic phthalides were isolated in moderate yields. Unfortunately, the enantiomeric excess was quite low compared to solution phase experiments.[45] Further studies are necessary to investigate these results. The introduction of Me_2Zn did not result in the desired product **13a**. To our knowledge, this is the first asymmetric addition of zinc reagents to polymer-bound aldehydes.

The third method involves a Sakurai-type addition of allyl silanes to resin **10**. The Sakurai reaction has seldom been applied to solid-phase synthesis. To our knowledge only

Table 4. Addition of primary amines to resins **10** and **11**

Resin	R^1	R^2	Product	Yield (%)
10	H	Me	**19**	90[a]
10	H	*n*Pr	**20b**	76[a]
10	H	Allyl	**20c**	77[a]
10	H	*c*Pr	**20d**	97[a]
10	H	*c*Hex	**20e**	82[a]
10	H	Bn	**20f**	75[b]
10	H	(*S*)-Phenylethyl	**20g**	22[b]
11	MeO	*n*Pr	**21b**	40[a]
11	MeO	Allyl	**21c**	17[a]
11	MeO	*c*Pr	**21d**	49[a]

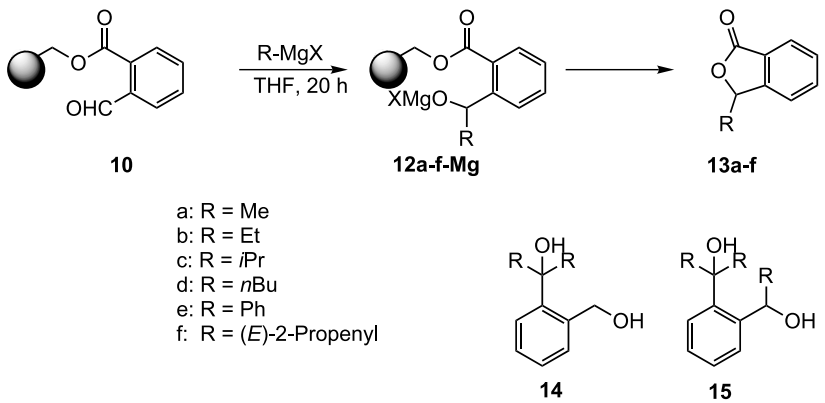
[a] Overall isolated yields of purified compounds. Purity of the crude mixture after cleavage determined by NMR-spectroscopy or GC >95%.
[b] Purity of the crude mixture not determined.

resin-bound allyl silanes have been used in similar studies.[47] Treatment of resin **10** with allyltrimethyl silanes in the presence of five equivalents of titanium tetrachloride the phthalides **13g–j**[48] were obtained in good yields and excellent purity. The Sakurai reaction was also accomplished with allyltrimethyl silanes and immobilized 6-formyl-2,3-dimethoxy benzoic acid (**11**), yielding the corresponding phthalides **18g–j** in good purities and moderate yields.

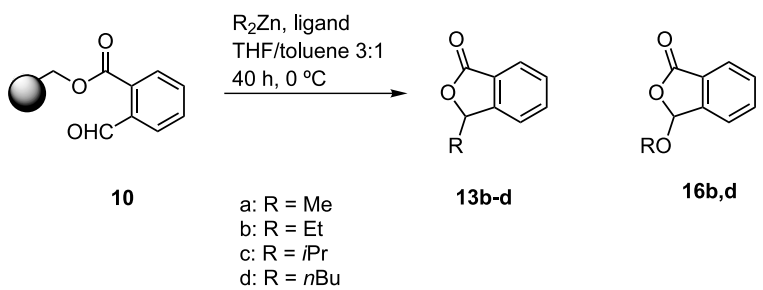
Primary amines were chosen as the next set of nucleophiles. In 2002, Ley and Taylor reported the introduction of *ortho*-carboxy benzaldehyde (**8**) and various primary amines in liquid phase synthesis of isoindolinones.[49] Reactions of resins **10** or **11** with primary amines produce 3-hydroxy-*N*-alkylisoindolinones **19**[50] or 3-alkylamino-*N*-alkylisoindolinones **20b–g** and **21b–d** with branched amines. Presumably, the cyclative cleavage of semiaminal **22** proceeds slower with sterically hindered amines leading to the formation of imines. The isoindoles **20** and **21** are interesting building blocks for further derivatization (Scheme 5).[51]

With these results in hand, we tried to interrupt the reaction on the intermediate immobilized imine **23b** and **23c** stage in order to add different nucleophiles. The addition of the *N*-nucleophile Et_2NH yielded excellent purities but low yields to the isoindolinones **25b** and **25c**. By addition of methanolate as nucleophile an isomerization of the double bond of the allyl moiety after cyclative-cleavage was observed. The isoindolinone **26** was isolated in an *E*/*Z*-ratio of 3:1 and purity higher than 98% was achieved after aqueous workup. However, no *C*-nucleophile reacted with the imine at different temperatures (up to room temperature). The cleavage with NaOMe of imine resins **23** which subsequently treated with Et_2Zn yielded in product (*E*)-**26**. This experiment shows that no reaction with Et_2Zn previously took place. In contrast, no product was found even after cleavage with NaOMe of the resins from the Grignard addition (Table 5).

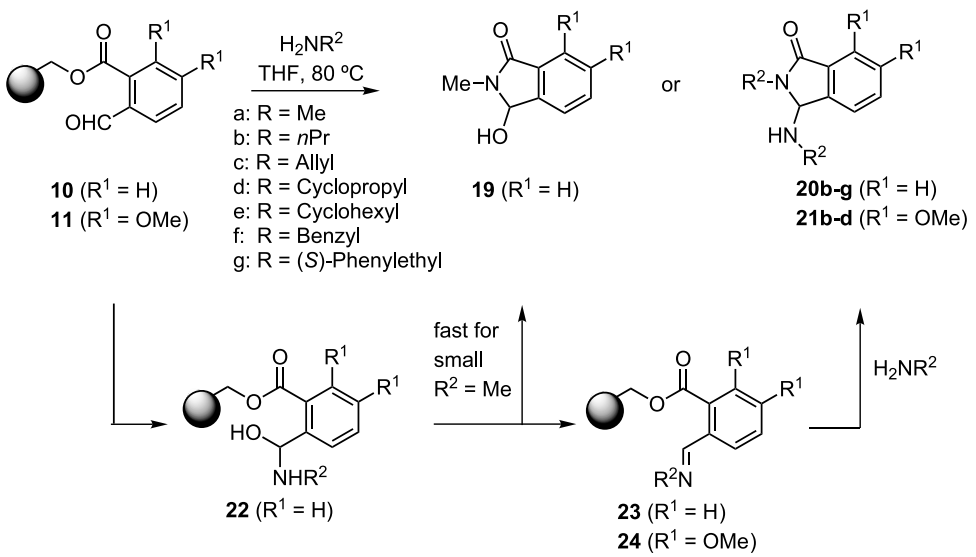
In conclusion, we presented the synthesis of phthalides via reaction of polymer-bound 2-formyl benzoic acids with different organometallic reagents (Grignard reagents, zinc reagents, allyl silanes). We also demonstrated that asymmetric induction was achieved by using chiral ligands. Isoindolinones, which represent an important scaffold for biologically active compounds are accessible from primary amines and different nucleophiles. Future work is dedicated to the application of more complex resins, their transformation on solid supports and the use of acylbenzoic acids.



Scheme 3. Solid-phase synthesis of 3-alkyl, 3-aryl and 3-alkenyl phthalides.

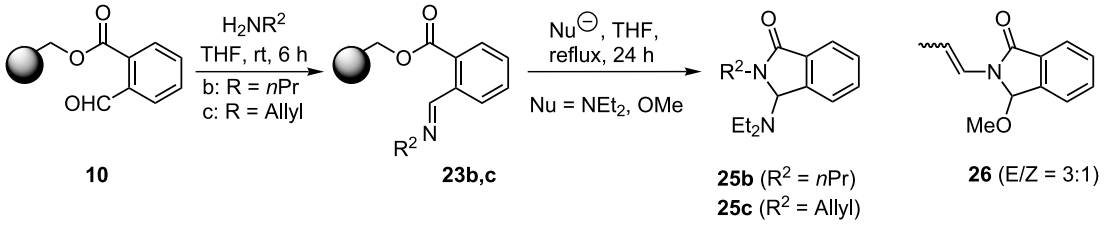


Scheme 4. Synthesis of phthalides using zinc reagents.



Scheme 5. Addition reactions of primary amines to form 3-hydroxy-isoindolinones **19** or 3-alkylamino-isoindolinones **20b–g** and **21b–d**.

Table 5. Addition of different nucleophiles to immobilized imine **23**



R^2NH_2	Nucleophile (NuX)	Product	Purity (%)[a]	Yield (%)[b]
nPrNH$_2$	Et$_2$NH	**25b**	94	10
AllylNH$_2$	Et$_2$NH	**25c**	92	12
AllylNH$_2$	MeONa	**26**	>98[c]	35[d]

[a] Purity of the crude mixture.
[b] After purification.
[c] After aqueous work-up.
[d] Combined yield of the *E*/*Z*-mixture.

2. Experimental

^{1}H NMR: Bruker DP 300 (300 MHz), Bruker DP 400 (400 MHz); δ=2.50 ppm for [D$_5$] dimethylsulfoxide, 3.31 ppm for [D$_3$] methanol, 7.24 ppm for CHCl$_3$. Description of signals: s=singlet, bs=broad singlet, d=doublet, t=triplet, q=quartet, m=multiplet, mc=centered multiplet, dd=doublet of doublet, ddd=doublet of dd, dt=doublet of triplets, dq=doublet of quartets, tt=triplet of triplets, ca=complex area. The spectra were analyzed according to first order. All couplings constants are absolute values. Abbreviations for signals: Ar-H=Ar. ^{13}C NMR: Bruker DP 300 (75 MHz), Bruker DP 400 (100 MHz); δ=39.52 ppm for perdeuterodimethylsulfoxide, 77.20 ppm for deuterochloroform, 49.00 for perdeuteromethanol. The signal structure was analyzed by DEPT and described as follows: +=primary or tertiary C-atom (positive signal), −=secondary C-atom (negative signal), and q=quaternary C-atom (no signal). IR (infrared-spectroscopy): Perkin Elmer FT-IR 1750. The substances were dissolved in distilled dichloromethane. The resins were measured as KBr pellets on a Bruker IFS88 IR. ps=polystyrene. MS (mass spectroscopy): EI-HRMS (electronic ionization—high resolution mass spectroscopy): Kratos MS 50 (70 eV) and Thermo Quest Finnigan MAT 95 XL (70 eV). GC (gas chromatography) analytical: Hewlett–Packard HP 5890 Series II 12 m×0.25 mm capillary column HP I (carrier gas N$_2$). HPLC: Varian WCOT fused silica 25 m×0.25 mm, coating CP chiral-dex CB DF=0.25. TLC (thin layer chromatography): Silica gel coated aluminium plates (Merck, silica gel 60, F$_{254}$). Detection under UV-light at 254 nm, displayed with molybdato phosphate (5% phosphor molybdic acid in ethanol, dipping solution), and potassium permanganate (0.45 g potassium permanganate and 2.35 g of sodium carbonate in 90 ml of water, dipping solution). Elemental analysis: elementar vario EL at the Mikroanalytisches Labor des Instituts für Organische Chemie der Universität Bonn. Reaction without nominated temperature were done at room temperature (rt). Solid materials, except resins, were powdered. All Chemicals, solvents, and reagents were purchased from Acros, Aldrich, Fluka, Janssen, or Merck. The Merrifield resin (1–2% cross-linked, 200–400 mesh) was obtained from Polymer-Laboratories with loading=1.06 g mol^{-1}. All resins were

washed sequentially by using a vacuum reservoir connected to a sintered glass frit. Cleavage was conducted using Teflon tubes with a frit connected to a vacuum line, with a glass pipette filled with glass wool, or were simply paper-filtered. Evaporation of the solvent was achieved using a rota-vapor and/or under a high vacuum (ca. 0.1 mbar). All solvents were dried by usual methods and distilled under argon. General washing procedure: (methanol, THF, pentane, dichloromethane) three times; (methanol, DMF, pentane, THF) once and (pentane, dichloromethane, pentane) two times.

2.1. General procedure for the attachment of benzoic acids to the merrifield resin

In a three-necked round bottom flask equipped with a mechanical stirrer, 9.70 g (30.0 mmol) of cesium carbonate was suspended in DMF and stirred for 10 min. Then, *ortho*-carboxybenzaldehyde **8** or **9** (30.0 mmol) was added and the mixture was once again stirred for 10 min. Afterwards, 10.0 g (10.6 mmol, loading 1.06 mmol/g) of Merrifield resin were added, and the mixture was stirred for 24 h at 50 °C. The mixture was allowed to reach room temperature. The resin was filtered off, washed with water, followed by the general washing procedure, and dried under high vacuum.

2.1.1. 2-Formylphenylcarboxymethyl polystyrene (10). IR (KBr): 3575 (m, ps), 3384 (vs), 3082 (m, ps), 3026 (m, ps), 2928 (s, ps), 2849 (m), 2761 (m), 2546 (s), 2254 (m), 1946 (m, ps), 1873 (m, ps), 1804 (m, ps), 1718 (vs, ps), 1596 (m), 1494 (m, ps), 1450 (m, ps), 1374 (m), 1268 (s, ps), 1193 (m, ps), 1132 (m), 1087 (m), 964 (m, ps), 907 (m, ps), 855 (m), 769 (m, ps), 706 (m, ps) cm^{-1}. Elemental analysis: found C: 88.01, H: 7.462.

2.1.2. 2-Formyl-5,6-dimethoxyphenylcarboxymethyl polystyrene (11). IR (KBr): 3573 (m, ps), 3060 (m, ps), 3026 (m, ps), 2926 (s, ps), 2761 (m), 2601 (m), 2337 (m), 1944 (s, ps), 1873 (m, ps), 1803 (m, ps), 1742 (vs, ps), 1600 (s), 1493 (s, ps), 1452 (m, ps), 1425 (m), 1372 (m), 1280 (m, ps), 1181 (m, ps), 1146 (m), 1053 (m), 967 (m, ps), 907 (m, ps), 841 (m), 816 (m), 762 (m, ps), 705 (s, ps) cm^{-1}. Elemental analysis: found C: 86.79, H: 7.478.

2.2. Synthesis of lactones 13a–j, 18 g,j

2.2.1. 1,2-Addition of organo lithium and organo magnesium reagents. The resin **10** was suspended in THF (20 ml/mmol resin), flushed with argon (crucial), and cooled to −70 or −10 °C. Five equivalents of the organo lithium or organo magnesium reagent were added. The reaction vial was occasionally shaken over a period of 20 h at constant temperature. The reaction was then quenched with 1 M HCl (aq.) The organic phase was separated, washed with water, and dried over $MgSO_4$. After removing the solvent under reduced pressure, the product was then purified by flash column chromatography on silica with *n*-pentane/diethyl ether (4:1) as eluent.

2.2.2. 1,2-Addition of organo zinc reagents promoted by achiral and chiral *N,O*-ligands. The resin **10** was suspended in THF (20 ml/mmol resin), flushed with argon, and cooled to 0 °C. Proportional to the amount of resin, 20 mol% of the *N,O*-ligand **17** or *N,N*-dimethylamino ethanol were dissolved in toluene (5 ml/mmol resin). After stirring for a few minutes, 5 equiv (related to the amount of resin) of the zinc reagent were added to the *N,O*-ligand solution. The zinc reagent/ligand-solution was stirred for one hour at room temperature and then added to the resin suspension at 0 °C. The reaction was quenched after 40 h at 0 °C with 1 M HCl (aq.). The organic phase was separated, washed with water, and dried over $MgSO_4$. After removing the solvent under reduced pressure, the product was purified by flash column chromatography on silica with *n*-pentane/diethylether (4:1) as the eluent.

2.3. Sakurai reaction

The resin **10** was suspended in THF (20 ml/mmol resin) and the mixture flushed with argon. Five equivalents of a 1 M solution of $TiCl_4$ in CH_2Cl_2 were added. After shaking for 2 h 5 equiv of allyl silane were added and the reaction mixture was shaken for 40 h at room temperature. Then, the reaction was quenched with a saturated aqueous $NaHCO_3$ solution. The aqueous phase was separated and extracted with diethyl-ether. The organic phase was washed with brine and dried with $MgSO_4$. After removing the solvent under reduced pressure, the product was purified by flash column chromatography on silica with *n*-pentane/diethyl-ether (4:1) as the eluent.

2.3.1. 3-Methyl-3*H*-isobenzofuran-1-one (13a). Yellow oil, 15% (from MeMgCl). ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.62 (d, ^3J=6.70 Hz, 3H, CH_3), 5.54 (q, ^3J=6.70 Hz, 1H, 3-H), 7.42 (dd, ^3J=7.58 Hz, ^4J=0.76 Hz, 1H, 5-H), 7.42 (tt, ^3J=8.21 Hz, ^4J=0.76 Hz, 1H, 4-H), 7.66 (dt, ^3J=7.46 Hz, ^4J=1.02 Hz, 1H, 6-H), 7.88 (d, ^3J=7.71 Hz, 1H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=20.6 (+, CH_3), 77.9 (+, C-3), 121.7 (+, C-6), 125.9 (+, C-4), 126.0 (q, C-7a), 129.2 (+, C-7), 134.2 (+, C-5), 151.4 (q, C-3a), 170.6 (q, C-1). IR (CH_2Cl_2): ν=1763 (CO), 1608 (arene) cm^{-1}. MS (EI), *m/z* (%)=148 (M^+, 25), 133 (60), 105 (100), 77 (35). HRMS ($C_9H_8O_2$): Calcd 148.0524, found 148.0533.

2.3.2. 3-Ethyl-3*H*-isobenzofuran-1-one (13b). Yellow oil, 21% (37% ee, from Et_2Zn). ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.98 (t, ^3J=7.3 Hz, 3H, CH_3), 1.81 (ddq, ^3J=14.6, 7.3, 7.3 Hz, 1H, CH_2), 2.10 (ddq, ^3J=14.6, 7.3, 4.4 Hz, 1H, CH_2), 5.43 (dd, ^3J=7.07, 4.4 Hz, 1H, 3-H), 7.41 (dd, ^3J=7.52 Hz, ^4J=0.82 Hz, 1H, 5-H), 7.50 (tt, ^3J=7.52 Hz, ^4J=0.76 Hz, 1H, 4-H), 7.65 (dt, ^3J=8.53 Hz, ^4J=1.07 Hz, 1H, 6-H), 7.88 (d, ^3J=7.70 Hz, 1H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=9.0 (+, CH_3), 27.9 (−, CH_2), 82.5 (+, C-3), 121.9 (+, C-6), 125.9 (+, C-4), 126.7 (q, C-7a), 129.2 (+, C-7), 134.1 (+, C-5), 149.3 (q, C-3a), 170.8 (q, C-1). IR (CH_2Cl_2): ν=1762 (CO), 1616 (arene) cm^{-1}. MS (EI), *m/z* (%)=162 (M^+, 15), 133 (100), 105 (25), 77 (10). HRMS ($C_{10}H_{10}O_2$): Calcd 162.0681, found 162.0672.

2.3.3. 3-Isopropyl-3*H*-isobenzofuran-1-one (13c). Yellow oil, 16% (from *i*PrMgCl), 27% (60% ee, from iPr_2Zn). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.79 (d, ^3J=6.79 Hz, 3H, CH_3), 1.11 (d, ^3J=6.97 Hz, 3H, CH_3), 2.26 (m, 1H, CH), 5.34 (d, ^3J=3.77 Hz, 1H, 3-H), 7.42 (dd, ^3J=7.54 Hz, ^4J=0.76 Hz, 1H, 5-H), 7.50 (t, ^3J=7.54 Hz, 1H, 4-H), 7.64 (dt, ^3J=7.35 Hz, ^4J=1.13 Hz, 1H, 6-H), 7.88 (d,

^3J=7.54 Hz, 1H, 7-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=15.9 (+, CH_3), 18.9 (+, CH_3), 32.6 (+, CH), 85.8 (+, C-3), 122.3 (+, C-6), 125.9 (+, C-4), 127.0 (q, C-7a), 129.2 (+, C-7), 134.0 (+, C-5), 149.1 (q, C-3a), 171.0 (q, C-1). IR (CH_2Cl_2): ν=1762 (CO), 1616 (arene) cm^{-1}. MS (EI), *m*/*z* (%)=176 (M^+, 15), 133 (100), 105 (20), 77 (10), 51 (5). HRMS ($C_{11}H_{12}O_2$): Calcd 176.0837, found 176.0840.

2.3.4. 3-*n*-Butyl-3*H*-isobenzofuran-1-one (13d). Yellow oil, 23% (from *n*BuMgCl, 31% (35% ee, from nBu_2Zn). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.89 (t, ^3J=7.16 Hz, 3H, CH_3), 1.31–2.10 (ca, 6H, 3×CH_2), 5.45 (dd, ^3J=7.71, 7.71, 4.15 Hz, 1H, 3-H), 7.41 (dd, ^3J=7.53 Hz, ^4J=0.75 Hz, 1H, 5-H), 7.50 (t, ^3J=7.53 Hz, 1H, 4-H), 7.64 (dt, ^3J=7.54 Hz, ^4J=1.13 Hz, 1H, 6-H), 7.87 (d, ^3J=7.73 Hz, 1H, 7-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.0 (+, CH_3), 22.2 (−, CH_2), 27.1 (−, CH_2), 34.7 (−, CH_2), 81.6 (+, C-3), 121.9 (+, C-6), 125.9 (+, C-4), 126.4 (q, C-7a), 129.2 (+, C-7), 134.1 (+, C-5), 150.3 (q, C-3a), 170.8 (q, C-1). IR (CH_2Cl_2): ν=1773 (CO), 1615 (arene) cm^{-1}. MS (EI), *m*/*z* (%)=190 (M^+, 5), 175 (5), 133 (100), 105 (20), 77 (10). HRMS ($C_{12}H_{14}O_2$): Calcd 190.0994, found 190.0991.

2.3.5. 3-Phenyl-3*H*-isobenzofuran-1-one (13e). Yellow oil, 13% (from PhMgCl). ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.29 (s, 1H, 3-H), 7.18–7.64 (ca, 8H, Ar-H), 7.86 (d, ^3J=7.58 Hz, 1H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=82.8 (+, C-3), 123.0 (+, C-6), 129.0 (+, C-4), 126.7 (q, C-7a), 128.5 (+, C-2′, C-6′), 129.1 (+, C-4′), 129.9 (+, C-3′, C-5′), 130.5 (+, C-7), 133.1 (+, C-5), 137.3 (q, C-1′), 149.8 (q, C-3a), 170.6 (q, C-1). IR (CH_2Cl_2): ν=1768 (CO), 1663 (arene) cm^{-1}. MS (EI), *m*/*z* (%)=210 (M^+, 90), 165 (40), 152 (20), 133 (15), 105 (100), 77 (45). HRMS ($C_{14}H_{10}O_2$): Calcd 210.0681, found 210.0688.

2.3.6. 3-Propenyl-3*H*-isobenzofuran-1-one (13f). Yellow oil, 15% (from (*E*)-CH_3CH=$CHMgCl$). ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.94 (dd, ^3J=7.07 Hz, ^4J=1.70 Hz, 3H, CH_3), 5.33 (m, 1H, CH=C*H*CH_3), 5.94 (m, 1H, C*H*=$CHCH_3$), 6.23 (d, ^3J=9.09 Hz, 1H, 3-H), 7.35 (m, ^3J=7.70, 0.88 Hz, 1H, 4-H), 7.51 (dd, ^3J=7.45, 7.45 Hz, 1H, 5-H), 7.64 (dd, ^3J=7.58 Hz, ^4J=1.14 Hz, 1H, 6-H), 7.89 (d, ^3J=7.71 Hz, 1H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=13.9 (+, CH_3), 77.1 (+, C-3), 122.5 (+, C-6), 125.8 (+, C-4), 126.0 (+, *C*H=$CHCH_3$), 126.2 (q, C-7a), 129.4 (+, C-7), 131.8 (+, CH=*C*HCH_3), 134.3 (+, C-5), 149.9 (q, C-3a), 170.8 (q, C-1). IR (CH_2Cl_2): ν=1763 (CO), 1606 (arene) cm^{-1}. MS (EI), *m*/*z* (%)=174 (M^+, 60), 159 (100), 146 (35), 133 (30), 105 (50), 77 (30), 51 (15). $C_{11}H_{10}O_2$ HRMS: Calcd 174.0681, found 174.0683.

2.3.7. 3-Allyl-3*H*-isobenzofuran-1-one (13g). Yellow oil, 73%. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.55–2.77 (ca, 2H, $(CH)_2CH_2$), 5.12 (dd, ^2J=5.46 Hz, ^3J=10.17 Hz, 1H, CH=C*H*H), 5.14 (dd, ^2J=5.46 Hz, ^3J=17.14 Hz, 1H, CH=CH*H*), 5.45 (dd, ^3J=5.93, 5.93 Hz 1H, 3-H), 6.72 (dddd, ^3J=16.95, 10.17, 7.16 Hz, 2H, C*H*=CH_2), 7.47 (m, 2H, 4-H, 5-H), 7.63 (dd, ^3J=7.53 Hz, ^4J=1.00 Hz, 1H, 6-H), 7.85 (d, ^3J=7.53 Hz, 1H, 7-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=38.8 (−, $(CH)_2CH_2$), 80.3 (+, C-3), 119.8 (−, CH=*C*H_2), 122.1 (+, C-6), 125.8 (+, C-4), 126.4 (q, C-7a), 129.3 (+, C-7), 131.3 (+, *C*H=CH_2), 134.1 (+, C-5), 149.5 (q, C-3a), 170.4 (q, C-1). IR (CH_2Cl_2): ν=1764 (CO), 1616 (arene), 999 (CH=CH_2) cm^{-1}. MS (EI), *m*/*z* (%)=174 (M^+, 10), 133 (100), 105 (15), 91 (5), 77 (20), 51 (5). HRMS ($C_{11}H_{10}O_2$): Calcd 174.0681, found 174.0685.

2.3.8. 3-(2-Acetoxymethyl)allyl-3*H*-isobenzofuran-1-one (13h). Yellow oil, 67%. ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.05 (s, 3H, CH_3), 2.55 (ddd, ^2J=15.03 Hz, ^3J=7.83 Hz, ^4J=0.63 Hz, 1H, CHC*H*H), 2.70 (ddd, ^3J=15.03, 4.99 Hz, ^4J=0.76 Hz, 1H, CHCH*H*), 4.52 (d, ^2J=13.25 Hz, 1H, C*H*HO), 4.58 (d, ^2J=13.25 Hz, 1H, CH*H*O), 5.13 (d, ^2J=0.82 Hz, 1H, C=C*H*H), 5.23 (d, ^2J=0.82 Hz, 1H, C=CH*H*), 5.59 (dd, ^3J=7.90, 4.99 Hz, 1H, C-3), 7.45 (dd, ^3J=7.50 Hz, ^4J=0.82 Hz, 1H, 4-H), 7.50 (ddd, ^3J=7.48, ^4J=0.76, 0.76 HZ, 1H, 5-H), 7.64 (dt, ^3J=7.48 Hz, ^4J=1.05 Hz, 1H, 6-H), 7.86 (d, ^3J=7.70 Hz, 1H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=21.0 (+, CH_3), 38.8 (−, CH*C*H_2), 67.0 (−, CH_2O), 79.4 (+, C-3), 117.7 (−, C=*C*H_2), 122.2 (+, C-6), 125.9 (+, C-4), 126.2 (q, C-7a), 129.5 (+, C-7), 134.1 (+, C-5), 138.4 (q, *C*=CH_2), 149.4 (q, C-3a), 170.2, 170.6 (q, O*C*OCH_3 and C-1). IR (CH_2Cl_2): ν=1765 (CO), 1615 (arene), 100 (CH=CH_2) cm^{-1}. MS (EI), *m*/*z* (%)=246 (M^+, 10), 220 (200), 133 (100), 105 (15), 91 (15), 77 (10), 55 (10).

2.3.9. 3-(2-Chloromethyl)allyl-3*H*-isobenzofuran-1-one (13i). Yellow oil, 69%. ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.59 (ddd, ^2J=9.60 Hz, ^3J=6.95 Hz, ^4J=1.01 Hz, 1H, CHC*H*H), 2.90 (ddd, ^2J=9.60 Hz, ^3J=4.60 Hz, ^4J=1.01 Hz, 1H, CHCH*H*), 4.02 (dd, ^2J=11.88 Hz, ^4J=0.88 Hz, 1H, ClC*H*H), 4.13 (dd, ^2J=11.88 Hz, ^4J=0.88 Hz, 1H, ClCH*H*), 5.14 (d, ^2J=0.76 Hz, 1H, C=C*H*H), 5.30 (d, ^2J=0.76 Hz, 1H, C=CH*H*), 5.62 (dd, ^3J=8.40, 4.369 Hz, 1H, C-3), 7.46–7.55 (ca, 2H, 4-H and 5-H), 7.66 (dt, ^3J=7.52 Hz, ^4J=1.09 Hz, 1H, 6-H), 7.87 (d, ^3J=7.71 Hz, 1H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=38.4 (−, CH*C*H_2), 48.5 (−, CH_2Cl), 79.5 (+, C-3), 119.2 (−, C=*C*H_2), 122.2 (+, C-6), 126.0 (+, C-4), 126.2 (q, C-7a), 129.6 (+, C-7), 134.2 (+, C-5), 139.7 (q, *C*=CH_2), 149.3 (q, C-3a), 170.2 (q, C-1). IR (CH_2Cl_2): ν=1767 (CO), 1614 (arene), 1003 (CH=CH_2) cm^{-1}. MS (EI), *m*/*z* (%)=222 (M^+, 20), 133 (100), 105 (15), 77 (10). HRMS ($C_{12}H_{11}ClO_2$): Calcd 222.0448, found 222.0452.

2.3.10. 3-(2-Trimethylsilyl-allyl)-3*H*-isobenzofuran-1-one (13j). Yellow oil, 51%. ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.12 (s, 9H, $Si(CH_3)_3$), 2.65 (dd, ^3J=7.83 Hz, ^2J=2.27 Hz, 1H, C*H*HCSi), 2.68 (dd, ^3J=7.83 Hz, ^2J=2.27 Hz, 1H, CH*H*CSi), 5.53–5.58 (ca, 2H, C-3 and CH_{cis}=CSi), 5.77 (d, ^2J=2.40 Hz, 1H, CH_{trans}=CSi), 7.44 (dd, ^3J=7.58 Hz, ^4J=0.99 Hz, 1H, 4-H), 7.50 (t, ^3J=7.58, 1H, 5-H), 7.62 (dd, ^3J=7.58 Hz, ^4J=0.99 Hz, 1H, 6-H), 7.87 (d, ^3J=7.58 Hz, 1H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=−1.3 (+, $Si(CH_3)_3$), 41,3 (−, CH*C*H_2), 80.3 (+, C-3), 122.4 (+, C-6), 125.9 (+, C-4), 126.4 (q, C-7a), 128.8 (−, C=*C*H_2), 129.3 (+, C-7), 133.9 (+, C-5), 146.5 (q, *C*=CH_2), 150.1 (q, C-3a), 170.5 (q, C-1). IR (CH_2Cl_2): ν=1758 (CO), 1616 (arene), 997 (CH=CH_2) cm^{-1}. MS (EI), *m*/*z* (%)=231 (M^+-CH_3, 100), 201 (10), 189 (10), 133 (80), 105 (20), 91 (10), 77 (15), 75 (30), 51 (15). HRMS ($C_{14}H_{18}O_2Si-CH_3$): Calcd 231.0841, found 231.0843.

2.3.11. 3-Allyl-6,7-dimethoxy-3*H*-isobenzofuran-1-one (18g). Yellow oil, 51%. ^{1}H NMR (400 MHz, $CDCl_3$):

δ=2.56 (ddd,, ^2J=2.40 Hz, ^3J=6.51, 7.45 Hz, 1H, CHC*H*HCH), 2.65 (ddd,, ^2J=2.40 Hz, ^3J=6.44, 6.57 Hz, 1H, CHCH*H*CH), 3.87 (s, 3H, CH_3O), 4.06 (s, 3H, CH_3O), 5.12 (dd, ^2J=4.42 Hz, ^3J=10.11 Hz, 1H, CH=CH*H*), 5.15 (dd, ^2J=4.49 Hz, ^3J=17.05 Hz, 1H, CH=C*H*H), 5.35 (dd, ^3J=6.07, 6.07 Hz 1H, 3-H), 5.72 (dddd, ^3J=6.51, 7.45, 10.11, 17.05 Hz, 1H, C*H*=CH_2), 7.02 (dd, ^3J=8.09 Hz, ^4J=0.76 Hz, 1H, 4-H), 7.18 (d, ^3J=8.15 Hz, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=39.2 (−, CH*C*H_2CH), 57.0, 62.5 (+, 2×CH_3O), 79.1 (+, C-3), 116.6 (+, C-4), 119.5 (+, C-5), 119.6 (−, CH=*C*H_2), 131.6 (+, *C*H=CH_2), 142.6 (q, C-3a), 148.5, 152.7 (q, C-6 and C-7), 167.9 (q, C-1). IR (CH_2Cl_2): ν=1761 (CO), 1599 (arene), 1014 (CH=CH_2) cm^{-1}. MS (EI), *m*/*z* (%)=234 (M^+, 10), 193 (100), 122 (10). HRMS ($C_{13}H_{14}O_4$): Calcd 234.0892, found 234.0897.

2.3.12. 3-(2-Chloromethyl-allyl)-6,7-dimethoxy-3*H*-isobenzofuran-1-one (18i). Yellow oil, 24%. ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.55 (ddd, ^2J=15.22 Hz, ^3J=8.37 Hz, ^4J=0.89 Hz, 1H, CHC*H*H), 2.84 (ddd, ^2J=15.22 Hz, ^3J=4.20 Hz, ^4J=0.89 Hz, 1H, CHCH*H*), 3.89 (s, 3H, CH_3O), 4.02 (dd, ^2J=11.88 Hz, ^4J=0.89 Hz, 1H, ClC*H*H), 4.08 (s, 3H, CH_3O), 4.13 (dd, ^2J=11.88 Hz, ^4J=0.89 Hz, 1H, ClCH*H*), 5.14 (d, ^2J=0.76 Hz, 1H, C=C*H*H), 5.30 (d, ^2J=0.76 Hz, 1H, C=CH*H*), 5.50 (ddd, ^3J=8.37, 4.20 Hz, ^4J=0.81 Hz, 1H, C-3), 7.06 (dd, ^3J=8.21 Hz, ^4J=0.81 Hz, 1H, 4-H), 7.20 (d, ^3J=8.21 Hz, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=38.8 (−, CH*C*H_2), 48.6 (−, CH_2Cl), 57.1, 62.5 (+, 2×CH_3O), 78.2 (+, C-3), 116.5 (+, C-4), 118.4 (q, C-7a), 119.1 (−, C=*C*H_2), 119.6 (+, C-5), 139.8 (q, *C*=CH_2), 142.4 (q, C-3a), 148.7, 152.9 (q, C-6 and C-7), 167.7 (q, C-1). IR (CH_2Cl_2): ν=1764 (CO), 1600 (arene), 1010 (CH=CH_2) cm^{-1}. MS (EI), *m*/*z* (%)=282 (M^+, 10), 256 (10), 193 (100). HRMS ($C_{14}H_{15}ClO_4$): Calcd 282.0659, found 222.0660.

2.3.13. 6,7-Dimethoxy-3-(2-trimethylsilanyl-allyl)-3*H*-isobenzofuran-1-one (18j). Yellow oil, 60%. ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.00 (s, 9H, $SiMe_3$), 2.50 (dd, ^3J=8.46 Hz, ^2J=3.16 Hz, 2H, CH_2CSi), 3.77 (s, 3H, CH_3O), 3.97 (s, 3H, CH_3O), 5.31 (t, ^3J=7.79 Hz, 1H, 3-H), 5.43 (dd, ^2J=2.27 Hz, ^4J=1.01 Hz, 1H, CH=CH*H*), 5.64 (dd, ^2J=2.27 Hz, ^4J=1.39 Hz, 1H, CH=C*H*H), 6.90 (dd, ^3J=8.21 Hz, ^4J=0.75 Hz, 1H, 4-H), 7.05 (dd, ^3J=8.21 Hz, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=1.3 (+, $Si(CH_3)_3$), 41.8 (−, $CHCH_2$), 57.2, 62.6 (+, 2×CH_3O), 79.0 (+, C-3), 116.8 (+, C-4), 118.6 (q, C-7a), 119.4 (+, C-5), 128.7 (−, C=*C*H_2), 143.4 (q, C-3a), 146.6 (q, *C*=CH_2), 148.7, 152.8 (q, C-6 and C-7), 167.9 (q, C-1). IR (CH_2Cl_2): ν=1760 (CO), 1601 (arene), 1045 (CH=CH_2) cm^{-1}. MS (EI), *m*/*z* (%)=306 (M^+, 10), 291 (10), 193 (100). HRMS ($C_{16}H_{22}O_4Si$): Calcd 306.1287, found 306.1294.

2.3.14. 3-Ethoxy-3*H*-isobenzofuran-1-one (16b). Yellow oil, 19% (from Et_2Zn). ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.31 (t, ^3J=7.07 Hz, 3H, CH_3), 3.85 (m, 1H, CH_2), 3.97 (m, 1H, CH_2), 6.35 (s, 1H, 3-H), 7.57 (ca, 2H, 4-H, 5-H), 7.64 (dt, ^3J=7.46 Hz, ^4J=1.14 Hz, 1H, 6-H), 7.87 (d, ^3J=7.54 Hz, 1H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=15.3 (+, CH_3), 66.1 (−, CH_2), 102.5 (+, C-3), 123.6 (+, C-6), 125.6 (+, C-4), 127.5 (q, C-7a), 130.9 (+, C-7), 134.5 (+, C-5), 145.3 (q, C-3a), 168.9 (q, C-1). IR (CH_2Cl_2): ν=1773 (CO), 1616 (arene) cm^{-1}. MS (EI), *m*/*z* (%)=178 (M^+, 20), 133 (100), 162 (20), 147 (10), 133 (100), 105 (25), 90 (10), 77 (10), 51 (5). HRMS ($C_{10}H_{10}O_3$): Calcd 178.0630, found 178.0634.

2.3.15. 3-Butoxy-3*H*-isobenzofuran-1-one (16d). Yellow oil, 19% (from nBu_2Zn). ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.92 (t, ^3J=7.39 Hz, 3H, CH_3), 1.36–1.70 (ca, 4H, 2×CH_2), 3.77 (ddd, ^2J=9.42 Hz, ^3J=6.69 Hz, ^2J=2.72 Hz, 1H, CH_2), 3.90 (ddd, ^2J=9.42 Hz, ^3J=6.50 Hz, ^2J=2.83 Hz, 1H, CH_2), 6.35 (s, 1H, 3-H), 7.56 (ca, 2H, 4-H, 5-H), 7.68 (dt, ^3J=7.48 Hz, ^4J=1.10 Hz, 1H, 6-H), 7.87 (d, ^3J=7.58 Hz, 1H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=13.9 (+, CH_3), 19.3 (−, CH_2), 31.7 (−, CH_2), 70.2 (−, CH_2), 102.7 (+, C-3), 123.6 (+, C-6), 125.6 (+, C-4), 127.5 (q, C-7a), 130.9 (+, C-7), 134.5 (+, C-5), 145.3 (q, C-3a), 168.9 (q, C-1). IR (CH_2Cl_2): ν=1763 (CO), 1608 (arene) cm^{-1}. MS (EI), *m*/*z* (%)=206 (M^+, 10), 175 (30), 162 (10), 149 (5), 133 (100), 105 (15), 77 (5). HRMS ($C_{12}H_{14}O_3$): Calcd 206.0943, found 206.0946.

2.3.16. 2-[2-(1-Hydroxy-ethyl)-phenyl]-propan-2-ol (15a). Yellow oil, 19% (from MeMgCl). ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.48 (d, ^3J=6.41 Hz, 3H, CHC*H*$_3$), 1.58 (s, 3H, CCH_3), 1.64 (s, 3H, CCH_3), 2.67 (bs, 1H, OH), 5.67 (q, ^3J=6.41 Hz, 3H, C*H*CH_3), 7.11–7.28 (ca, 3H, Ar-H), 7.52 (dd, ^3J=7.72 Hz, ^4J=1.60 Hz, 1H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=23.9 (+, CH_3), 32.7 (+, CH_3), 32.9 (+, CH_3), 66.6 (+, CH), 125.7, 127.1, 127.3, 127.7 (+, C-Ar), 143.9, 144.8 (q, C-Ar). IR (CH_2Cl_2): ν=3596, 3411 (OH), 1605 (arene) cm^{-1}.

2.3.17. 3-(2-Hydroxymethyl-phenyl)-pentan-3-ol (14b). Yellow oil, 17% (from EtMgCl). ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.78 (t, ^3J=7.45 Hz, 6H, 2×CH_3), 1.75–2.00 (m, 4H, 2×C*H*$_2$$CH_3$), 2.75 (s, 2H, 2×OH), 4.77 (s, 2H, CH_2OH), 7.10–7.25 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=8.2 (+, 2×CH_3), 36.7 (−, 2×C*H*$_2$$CH_3$), 66.8 (−, CH_2), 73.5 (q, *C*(CH_2CH_3)$_2$), 126.8, 127.9, 128.1, 132.3 (+, C-Ar), 139.3 (q, *C*-$_{Ar}$$CH_2OH$), 143.5 (q, C_{Ar}C(CH_2CH_3)$_2$). IR (CH_2Cl_2): ν=3598, 3472 (OH), 1602 (arene) cm^{-1}. $C_{12}H_{18}O_2$.

2.3.18. 3-[2-(1-Hydroxy-propyl)-phenyl]-pentan-3-ol (15b). Yellow oil, 11% (from EtMgCl). ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.79 (t, ^3J=7.45 Hz, 6H, C(CH_2C*H*$_3$)$_2$), 0.99 (t, ^3J=7.45 Hz, 3H, $CHCH_2$C*H*$_3$), 1.76–2.14 (ca, 6H, 3×CH_2), 5.43 (t, ^3J=7.07 Hz, 1H, 3-H), 7.25–7.67 (ca, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=8.3 (+, C(CH_2*C*H$_3$)$_2$), 9.9 (+, $CHCH_2$*C*H$_3$), 27.9 (−, CH*C*H_2CH_3), 36.8 (−, C(*C*H_2CH_3)$_2$), 66.8 (+, CHOH), 82.5 (q, COH), 121.9, 125.9, 129.2, 132.3 (+, C-Ar), 139.4, 150.0 (q, C-Ar). IR (CH_2Cl_2): ν=3597, 3410 (OH), 1605 (arene) cm^{-1}. MS (EI), *m*/*z* (%)=222 (M^+, 5), 220 (10), 162 (10), 147 (100), 133 (45), 105 (45), 77 (20), 59 (39).

2.3.19. 5-(2-Hydroxymethyl-phenyl)-nonan-5-ol (14d). Yellow oil, 27% (from *n*BuMgCl). ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.76 (t, ^3J=7.20 Hz, 6H, 2×CH_3), 0.90–1.30 (ca, 8H C*H*$_2$C*H*$_2$$CH_3$), 1.70, 1.88 (m, 4H, 2×CC*H*2CH2), 3.20 (bs, 1H, OH), 4.71 (s, 2H, C*H*$_2$OH), 7.06–7.21 (ca, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=14.2 (+, 2×CH_3), 23.2 (−, 2×*C*H_2CH_3), 26.0 (−, 2×CH_2*C*H_2CH_2), 44.5 (−,

2×CCH_2CH_2), 66.7 (−, CH_2OH), 80.4 (q, COH), 126.7, 127.8, 128.1, 132.2 (+, C-Ar), 138.9, 144.3 (q, C-Ar). IR (CH_2Cl_2): ν=3591, 3488 (OH), 1602 (arene) cm^{-1}.

2.4. Synthesis of the isoindolinones 19, 20b–g and 21b–c

The resin **10** or **11** was suspended in THF (20 ml/mmol resin) and flushed with argon. Five equivalents of the primary amine were added and the reaction mixture was shaken and refluxed for 48 h. The liquid phase was separated. The solvent and the excess of amine was removed under reduced pressure or high vacuum. The product was purified by flash column chromatography on silica with *n*-pentane/diethylether (1:1 for **19** and **20**, 1:3 for **21** and 9:1 for **20g**) as the eluent.

2.5. Synthesis of the isoindolinones 25b,c and 26

The resin **10** was suspended in THF (20 ml/mmol resin) and flushed with argon. Two equivalents of the primary amines allyl amine or *n*-propyl amine were added and the reaction mixture was shaken for 6 h at room temperature. The resins were filtered off, washed with water, followed by the general washing procedure, and dried under high vacuum. The resin was then suspended again in THF (20 ml/mmol resin) and flushed with argon. Two equivalents of diethyl amine or NaOMe were added, and the reaction mixture was shaken and refluxed for 24 h. The liquid phase was separated. The solvent and the excess of amine was removed under reduced pressure or high vacuum. The products were purified by flash column chromatography on silica with *n*-pentane/diethyl ether (2:1) as the eluent.

2.5.1. 3-Hydroxy-2-methyl-2,3-dihydro-isoindol-1-one (19). Colorless powder, 78%. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.81 (s, 3H, CH_3), 4.55 (bs, 1H, OH), 5.52 (s, 1H, 3-H), 7.28–7.54 (ca, 4H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=26.2 (+, CH_3), 83.7 (+, CH), 123.0 (+, C-4), 123.3 (+, C-7), 129.6 (+, C-5), 131.5 (q, C-7a), 132.2 (+, C-6), 144.1 (q, C-3a), 167.8 (q, CON). IR (CH_2Cl_2): ν=3333 (OH), 1694 (CON), 1619 (arene) cm^{-1}. MS (EI), *m/z* (%)=162 (M^-, 100), 146 (80), 133 (35), 105 (55), 91 (30), 77 (50). HRMS ($C_9H_9NO_2$): Calcd 163.0633, found 163.0639.

2.5.2. 2-Propyl-3-propylamino-2,3-dihydro-isoindol-1-one (20b). Colorless powder, 76%. ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.79 (t, ^3J=7.32 Hz, 3H, CH_3), 0.92 (t, ^3J=7.42 Hz, 3H, CH_3), 1.34 (m, 2H, $CH_3CH_2CH_2NH$), 1.62 (m, 2H, $CH_3CH_2CH_2N$), 1.98 (ddd, ^3J=14.31, 7.25 Hz, ^2J=4.52 Hz, 1H, NHC*H*H), 2.21 (ddd, ^3J=13.94, 6.99 Hz, ^2J=4.52 Hz, 1H, NHCH*H*), 3.08 (ddd, ^3J=13.90, 8.28 Hz, ^2J=5.39 Hz, 1H, NC*H*H), 3.76 (ddd, ^3J=13.75, 8.48 Hz, ^2J=5.39 Hz, 1H, NCH*H*), 5.35 (s, 1H, 3-H), 7.38–7.51 (ca, 3H, Ar-H), 7.75 (dd, ^3J=7.54 Hz, ^4J=0.88 Hz, 1H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=11.5, 11.7 (+, 2×CH_3), 21.9, 23.5 (−, 2×CH_2CH_3), 40.6 (−, NCH_2), 43.0 (−, $NHCH_2$), 72.7 (+, C-3), 123.1 (+, C-4), 123.2 (+, C-7), 129.0 (+, C-5), 131.5 (+, C-6), 133.2 (q, C-7a), 143.7 (q, C-3a), 167.8 (q, CON). IR (CH_2Cl_2): ν=3361 (NH), 1694 (CON), 1617 (arene) cm^{-1}. MS (EI), *m/z* (%)=232 (M^+, 20), 220 (25), 205 (50), 174 (100), 132 (40), 104 (5), 77 (5). HRMS ($C_{14}H_{20}N_2O$): Calcd 232.1576, found 232.1580.

2.5.3. 2-Allyl-3-allylamino-2,3-dihydro-isoindol-1-one (20c). Yellow oil, 77%. ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.00 (bs, 1H, NH), 2.73 (ddt, ^2J=14.15 Hz, ^3J=5.43 Hz, ^4J=3.24 Hz, 1H, NHC*H*H), 2.90 (ddt, ^2J=14.15 Hz, ^3J=6.31 Hz, ^4J=2.80 Hz, 1H, NHCH*H*), 3.76 (ddt, ^2J=15.54 Hz, ^3J=7.07 Hz, ^4J=2.49 Hz, 1H, NC*H*H), 4.49 (ddt, ^2J=15.54 Hz, ^3J=4.93 Hz, ^4J=3.06 Hz, 1H, NCH*H*), 4.98 (ddd, ^3J=10.23 Hz, ^4J=2.80 Hz, ^2J=1.58 Hz, 1H, CH=C*H*H), 5.07 (ddd, ^3J=17.18 Hz, ^4J=3.24 Hz, ^2J=1.58 Hz, 1H, CH=CH*H*), 5.16 (ddd, ^3J=10.10 Hz, ^4J=2.49 Hz, ^2J=1.64 Hz, 1H, CH=C*H*H), 5.20 (ddd, ^3J=17.18 Hz, ^4J=3.06 Hz, ^2J=1.64 Hz, 1H, CH=CH*H*), 5.37 (s, 1H, 3-H), 5.69–5.89 (ca, 2H, 2×CH_2CH=CH_2), 7.42–7.52 (ca, 3H, Ar-H), 7.75 (dd, ^3J=7.33 Hz, ^4J=1.01 Hz, 1H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=42.0 (−, NCH_2), 44.2 (−, $NHCH_2$), 72.3 (+, C-3), 116.1, 117.8 (−, 2×CH=CH_2), 123.3 (+, C-4), 123.4 (+, C-7), 129.2 (+, C-5), 131.8 (+, C-6), 132.9 (q, C-7a), 133.5 (+, C*H*=CH_2), 136.3 (+, C*H*=CH_2), 143.4 (q, C-3a), 167.5 (q, CON). IR (CH_2Cl_2): ν=3374 (NH), 1694 (CON), 1617 (arene) cm^{-1}. MS (EI), *m/z* (%)=228 (M^+, 5), 220 (10), 205 (30), 172 (100), 130 (20). HRMS ($C_{14}H_{16}N_2O$): Calcd 228.1263, found 228.1269.

2.5.4. 2-Cyclopropyl-3-cyclopropylamino-2,3-dihydro-isoindol-1-one (20d). Colorless powder, 97%. ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.15–0.84 (ca, 8H, 4×CH_2), 2.06 (dd, ^3J=8.97, 4.29 Hz, 1H, C*H*NH), 2.55 (bs, 1H, NH), 2.66 (dd, ^3J=8.59, 4.43 Hz, 1H, C*H*N), 5.17 (s, 1H, 3-H), 7.34–7.47 (ca, 3H, Ar-H), 7.70 (d, ^3J=7.33 Hz, 1H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=5.0, 5.4, 7.0, 8.1 (−, 4×CH_2), 22.9, 24.7 (+, 2×CH), 74.7 (+, C-3), 123.1 (+, C-4), 123.6 (+, C-7), 128.9 (+, C-5), 131.5 (+, C-6), 132.9 (q, C-7a), 143.7 (q, C-3a), 168.9 (q, CON). IR (CH_2Cl_2): ν=3356 (NH), 1695 (CON), 1618 (arene) cm^{-1}. MS (EI), *m/z* (%)=172 ($M-C_3H_6N^+$, 100), 145 (20), 132 (15), 115 (10).

2.5.5. 2-Cyclohexyl-3-cyclohexylamino-2,3-dihydro-isoindol-1-one (20e). Colorless powder, 82%. ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.91–1.95 (ca, 20H, 10×CH_2), 2.62 (m, 1H, C*H*NH), 3.70 (m, 1H, C*H*N), 5.39 (s, 1H, 3-H), 7.38–7.51 (ca, 3H, Ar-H), 7.74 (d, ^3J=7.32 Hz, ^4J=1.01 Hz, 1H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=24.9, 25.2, 25.8, 26.0, 26.5, 26.6 (−, 6×CH_2), 30.8, 31.1 (−, 4×CH_2), 52.0, (+, CHN), 53.2 (+, CHNH), 72.3 (+, C-3), 123.2 (+, C-4), 125.7 (q, C-7a), 128.9 (+, C-7), 131.4 (+, C-6), 133.4 (+, C-5), 145.4 (q, C-3a), 167.8 (q, CON). IR (CH_2Cl_2): ν=3374 (NH), 1685 (CON), 1616 (arene) cm^{-1}. MS (EI), *m/z* (%)=312 (M^+, 10), 220 (10), 214 (100), 205 (30), 133 (15), 132 (70), 105 (10), 77 (5), 55 (5). HRMS ($C_{20}H_{28}N_2O$): calcd 312.2202, found 312.2200.

2.5.6. 2-Benzyl-3-benzylamino-2,3-dihydro-isoindol-1-one (20f). Yellow oil, 75%. ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.00 (bs, 1H, NH), 3.18 (d, ^2J=13.01 Hz, 1H, C*H*HNH), 3.29 (d, ^2J=13.01 Hz, 1H, CH*H*NH), 4.27 (d, ^2J=14.97 Hz, 1H, C*H*HN), 5.09 (d, ^2J=14.97 Hz, 1H, CH*H*N), 5.25 (s, 1H, 3-H), 7.06–7.84 (ca, 14H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=43.4 (−, CH_2N), 45.8 (−, CH_2NH), 72.5 (+, C-3), 123.3 (+, C-4), 123.7 (+, C-7), 127.5 (+, CH_{Ar}), 127.8 (+, CH_{Ar}), 128.2 (+, 2×CH_{Ar}), 128.4 (+, 2×CH_{Ar}), 128.6 (+, 2×CH_{Ar}), 129.0 (+, 2×CH_{Ar}), 129.3 (+, C-6), 132.0 (+, C-5), 133.0 (q, C-7a), 137.5 (q, CH_2C_{Ar}), 139.8

(q, CH_2C_{Ar}), 143.5 (q, C-3a), 167.9 (q, CON). IR (CH_2Cl_2): ν=1693 (CON), 1615 (arene), 918 (arene) cm^{-1}. MS (EI), *m/z* (%)=328 (M^+, 15), 237 (15), 222 (95), 193 (10), 132 (10), 106 (30), 91 (100). HRMS ($C_{22}H_{20}N_2O$): Calcd 328.1576, found 328.1569.

2.5.7. (*S*,*S*)-2-(1-Phenyl-ethyl)-3-(1-phenyl-ethylamino)-2,3-dihydro-isoindol-1-one (20g). Colorless oil, 22%, 1:1-mixture of diastereomers. ^1H NMR (300 MHz, $CDCl_3$): δ=0.81, 1.08 (, ^3J=6.59, 6.59 Hz, 3H, NHCHCH_3), 1.79, 1.83 (d, ^3J=7.16, 7.16 Hz, 3H, NCHCH_3), 3.61, 3.72 (q, ^3J=6.59, 6.59 Hz, 3H, NHC*H*CH_3), 5.02, 5.28 (s, 1H, 3-H), 5.33, 5.62 (q, ^3J=7.16, 7.16 Hz, 1H, NC*H*CH_3), 6.93–7.47, 7.67–7.77 (ca, 14H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=18.1, 18.4 (+, NCH*C*H_3), 23.8, 24.6 (+, NHCH*C*H_3), 50.5, 50.7 (+, NCH), 52.5, 55.2 (+, NHCH), 71.5, 72.0 (+, C-3), 123.0–131.8 (+, CH_{Ar}), 132.1, 132.6, 141.1, 142.3, 144.0, 145.7, 146.0, 146.1 (q, C_{Ar}), 167.6 and 168.2 (q, CON). IR (CH_2Cl_2): ν=2923 (aliphatic), 1686 (CON), 1602 (arene) cm^{-1}. MS (EI), *m/z* (%)=356 (M^+, 15), 341 (15), 236 (20), 225 (15), 172 (100), 132 (25), 105 (20), 77 (10). HRMS ($C_{24}H_{24}N_2O$): Calcd 356.1889, found 356.1886.

2.5.8. 6,7-Dimethoxy-2-propyl-3-propylamino-2,3-dihydro-isoindol-1-one (21b). Yellow oil, 40%. ^1H NMR (300 MHz, $CDCl_3$): δ=0.80 (t, ^3J=7.44 Hz, 3H, CH_3), 0.94 (t, ^3J=7.44 Hz, 3H, CH_3), 1.33 (m, 2H, $CH_3CH_2CH_2NH$), 1.61 (m, 2H, $CH_3CH_2CH_2N$), 1.80 (bs, 1H, NH), 2.01 (ddd, ^3J=14.32, 7.22 Hz, ^2J=4.34 Hz, NHC*H*H), 2.23 (ddd, ^3J=14.04, 7.07 Hz, ^2J=4.43 Hz, 1H, NHCH*H*), 3.05 (ddd, ^3J=13.85, 8.29 Hz, ^2J=5.56 Hz, 1H, NC*H*H), 3.73 (ddd, ^3J=13.85, 8.56 Hz, ^2J=5.18 Hz, 1H, NCH*H*), 3.86 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 5.23 (s, 1H, 3-H), 7.04 (d, ^3J=8.10 Hz, 1H, 4-H), 7.13 (d, ^3J=8.10 Hz, 1H, 5-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=11.7, 11.9 (+, 2×CH_2CH_3), 21.8, 23.7 (−, 2×CH_2CH_3), 40.8 (−, NCH_2), 42.9 (−, $NHCH_2$), 56.8, 62.6 (+, 2×OCH_3), 71.7 (+, C-3), 116.1 (+, C-4), 118.2 (+, C-5), 125.1 (q, C-7a), 137.1 (q, C-3a), 147.1 (q, C-7), 153.2 (q, C-6), 16708 (q, CON). IR (CH_2Cl_2): ν=2926 (aliphatic), 1687 (CON). 1613 (arene) cm^{-1}. MS (EI), *m/z* (%)=292 (M^+, 5), 234 (10), 220 (25), 205 (100), 145 (5). HRMS ($C_{16}H_{24}N_2O_3$): Calcd 292.1787, found 292.1793.

2.5.9. 2-Allyl-3-allylamino-6,7-dimethoxy-2,3-dihydro-isoindol-1-one (21c). Colorless oil, 49%. ^1H NMR (400 MHz, $CDCl_3$): δ=1.95 (bs, 1H, NH), 2.77 (ddt, ^2J=14.15 Hz, ^3J=5.43 Hz, ^4J=3.16 Hz, 1H, NHC*H*H), 2.90 (ddt, ^2J=14.15 Hz, ^3J=6.44 Hz, ^4J=2.59 Hz, 1H, NHCH*H*), 3.73 (ddt, ^2J=15.60 Hz, ^3J=7.14 Hz, ^4J=2.02 Hz, 1H, NC*H*H), 3.86 (s, 3H, OCH_3), 4.05 (s, 6H, OCH_3), 4.45 (ddt, ^2J=15.60 Hz, ^3J=5.00 Hz, ^4J=3.07 Hz, 1H, NCH*H*), 4.99 (ddd, ^3J=10.24 Hz, ^4J=4.30 Hz, ^2J=1.59 Hz, 1H, CH=C*H*H), 5.08 (ddd, ^3J=17.18 Hz, ^4J=4.93 Hz, ^2J=1.59 Hz, 1H, CH=CH*H*), 5.16 (ddd, ^3J=10.17 Hz, ^4J=3.79 Hz, ^2J=1.40 Hz, 1H, CH=C*H*H), 5.21 (ddd, ^3J=17.18 Hz, ^4J=4.43 Hz, ^2J=1.40 Hz, 1H, CH=CH*H*), 5.27 (s, 1H, 3-H), 5.67–5.89 (ca, 2H, 2×CH_2CH=CH_2), 7.05 (d, ^3J=8.08 Hz, 1H, 4-H), 7.16 (d, ^3J=8.08 Hz, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=42.1 (−, NCH_2), 44.0 (−, $NHCH_2$), 56.8, 62.6 (+, 2×OCH_3), 71.3 (+, C-3), 116.0 (−, CH=CH_2), 116.4 (+, C-4), 117.8 (−, CH=CH_2), 118.5 (+, C-5), 124.8 (q, C-7a), 133.6 (+, C*H*=CH_2), 136.7 (+, C*H*=CH_2), 136.7 (q, C-3a), 147.2 (q, C-7), 153.4 (q, C-6), 167.7 (q, CON). IR (CH_2Cl_2): ν=3373 (NH), 1694 (CON), 1599 (arene), 1045 (CH_2=CH) cm^{-1}. MS (EI), *m/z* (%)=288 (M^+, 15), 247 (10), 232 (100), 205 (5), 190 (10). HRMS ($C_{16}H_{20}N_2O_3$): Calcd 288.1474, found 288.1475.

2.5.10. 2-Cyclopropyl-3-cyclopropylamino-6,7-dimethoxy-2,3-dihydro-isoindol-1-one (21d). Colorless oil, 17%. ^1H NMR (400 MHz, $CDCl_3$): δ=0.15–0.42 (ca, 4H, 2×CH_2), 0.68–1.05 (ca, 5H, 2×CH_2 and NH), 2.08 (dd, ^3J=9.35, 4.92 Hz, 1H, C*H*NH), 2.64 (dd, ^3J=6.95, 4.04 Hz, 1H, C*H*N), 3.85 (s, 3H, OCH_3), 4.05 (s, 6H, OCH_3), 5.12 (s, 1H, 3-H), 7.02 (d, ^3J=8.08 Hz, 1H, 4-H), 7.11 (d, ^3J=8.08 Hz, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=5.1, 5.5, 7.2, 8.0 (−, 4×CH_2), 23.1, 24.7 (+, 2×CH), 56.9, 62.6 (+, 2×OCH_3), 73.6 (+, C-3), 116.3 (+, C-4), 118.9 (+, C-5), 125.0 (q, C-7a), 136.5 (q, C-3a), 147.0 (q, C-7), 153.3 (q, C-6), 167.2 (q, CON). IR (CH_2Cl_2): ν=3357 (NH), 1694 (CON), 1600 (arene) cm^{-1}. MS (EI), *m/z* (%)=232 ($M-C_3H_6N^+$, 100), 190 (10), 84 (30). $C_{16}H_{20}N_2O_3$.

2.5.11. 3-Diethylamino-2-propyl-2,3-dihydro-isoindol-1-one (25b). Colorless oil, 10%. ^1H NMR (400 MHz, $CDCl_3$): δ=0.94 (t, ^3J=7.46 Hz, 3H, CH_3), 1.01–1.12 (ca, 6H, 2×CH_3), 1.57 (m, 2H, $CH_3CH_2CH_2NH$), 2.56 (m, 4H, $N(CH_2CH_3)_2$), 3.21 (ddd, ^3J=12.88, 7.07 Hz, ^2J=6.44 Hz, 1H, NC*H*H), 3.78 (ddd, ^3J=13.13, 7.90 Hz, ^2J=6.44 Hz, 1H, NCH*H*), 5.34 (s, 1H, 3-H), 7.40–7.51 (ca, 3H, Ar-H), 7.80 (d, ^3J=6.94 Hz, 1H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=11.7 (+, $CH_2CH_2CH_3$), 15.0 (+, $N(CH_2CH_3)_2$), 21.9 (−, $CH_2CH_2CH_3$), 38.4, 41.4, 43.7 (−, 3×NCH_2), 76.1 (+, C-3), 123.4 (+, C-4), 123.5 (+, C-7), 128.9 (+, C-5), 131.2 (+, C-6), 133.5 (q, C-7a), 143.9 (q, C-3a), 167.7 (q, CON). IR (CH_2Cl_2): ν=2925 (aliphatic), 1686 (CON), 1615 (arene) cm^{-1}. MS (EI), *m/z* (%)=246 (M^+, 10), 174 (100), 146 (10), 132 (30), 104 (5), 77 (5). $C_{15}H_{22}N_2O$, HRMS: Calcd 246.1732, found 246.1740.

2.5.12. 2-Allyl-3-diethylamino-2,3-dihydro-isoindol-1-one (25c). Colorless oil, 12%. ^1H NMR (400 MHz, $CDCl_3$): δ=0.90–1.10 (ca, 6H, 2×CH_2CH_3), 2.45–2.60 (ca, 4H, 2×CH_2CH_3), 3.75 (dd, ^2J=13.90 Hz, ^3J=6.82 Hz, 1H, NC*H*H), 4.50 (d, ^2J=13.90 Hz, 1H, NCH*H*), 5.30 (s, 1H, 3-H), 5.13 (m, 2H, CH=CH_2), 5.77 (dddd, ^3J=17.37, 10.42, 7.32, 6.82 Hz, 1H, C*H*=CH_2), 7.35–7.49 (ca, 3H, Ar-H), 7.77 (d, ^3J=7.07 Hz, 1H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=15.1 (+, 2×CH_3), 42.3 (−, NCH_2CH), 43.8 (−, 2×CH_2CH_3), 75.9 (+, C-3), 117.3 (−, CH=CH_2), 123.5 (+, C-4), 123.6 (+, C-7), 128.9 (+, C-5), 131.4 (q, C-7a), 133.2 (+, C-6), 133.8 (+, C*H*=CH_2), 143.5 (q, C-3a), 170.0 (q, CON). IR (CH_2Cl_2): ν=2922 (aliphatic), 1693 (CON), 1615 (arene) cm^{-1}. MS (EI), *m/z* (%)=244 (M^+, 10), 172 (100), 152 (15), 132 (15), 77 (5). HRMS ($C_{15}H_{20}N_2O$): Calcd 244.1576, found 244.1579.

2.5.13. 3-Methoxy-2-propenyl-2,3-dihydro-isoindol-1-one ((*E*)-26). Colorless solid, 25%. ^1H NMR (400 MHz, $CDCl_3$): δ=1.78 (dd, ^3J=6.75 Hz, ^4J=1.67 Hz, 2H, CH=CHCH_2), 2.84 (s, 1H, OCH_3), 5.66 (dt, ^3J=14.65, 6.75 Hz, 1H, CH=$CH_{trans}CH_2$), 6.09 (s, 1H, 3-H), 6.92 (dd, ^3J=14.65 Hz, ^4J=1.67 Hz, 1H, NC*H*=CH), 7.53 (d,

^3J=8.35 Hz, 1H, 4-H), 7.54 (d, ^3J=7.39 Hz, 1H, 7-H), 7.59 (dd, ^3J=7.39 Hz, ^4J=1.21 Hz, 1H, 6-H), 7.83 (dd, ^3J=8.35 Hz, ^4J=1.21 Hz, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=16.0 (+, CH*C*H$_3$), 48.7 (+, OCH$_3$), 86.2 (+, C-3), 109.1 (+, CH$_2$=*C*HCH$_3$), 122.4 (+, N*C*H=CH), 123.6 (+, C-4), 123.9 (+, C-7), 130.4 (+, C-5), 132.6 (q, C-7a), 132.9 (+, C-6), 140.5 (q, C-3a), 165.4 (q, CON). IR (CH_2Cl_2): ν=2920 (aliphatic), 1710 (CON), 1615 (arene) cm^{-1}. MS (EI), *m/z* (%)=203 (M$^+$, 80), 188 (80), 172 (100), 161 (15), 143 (15), 132 (25), 97 (20), 77 (15), 57 (30). HRMS ($C_{12}H_{13}NO_2$): Calcd 203.0946, found 203.0950.

2.5.14. 3-Methoxy-2-(*Z*)-propenyl-2,3-dihydro-isoindol-1-one ((*Z*)-26). Colorless solid, 10%. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.84 (dd, ^3J=7.17 Hz, ^4J=1.69 Hz, 2H, CH=CHC*H*$_2$), 2.91 (s, 1H, OCH$_3$), 5.34 (dt, ^3J=9.13, 7.17 Hz, 1H, CH=C*H*$_{cis}$CH$_2$), 6.19 (s, 1H, 3-H), 6.33 (dd, ^3J=9.13 Hz, ^4J=1.69 Hz, 1H, NC*H*=CH), 7.52 (d, ^3J=6.95 Hz, 1H, 4-H), 7.55 (d, ^3J=7.20 Hz, 1H, 7-H), 7.59 (dd, ^3J=8.97, 7.20 Hz, 1H, 6-H), 7.84 (dd, ^3J=8.97, 6.95 Hz, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=13.5 (+, CH*C*H$_3$), 49.6 (+, OCH$_3$), 87.3 (+, C-3), 117.0 (+, CH$_2$=*C*HCH$_3$), 121.0 (+, N*C*H=CH), 123.7 (+, C-4), 124.0 (+, C-7), 130.4 (+, C-5), 132.6 (q, C-7a), 132.8 (+, C-6), 140.3 (q, C-3a), 166.1 (q, CON). IR (CH_2Cl_2): ν=2922 (aliphatic), 1710 (CON), 1616 (arene) cm^{-1}. MS (EI), *m/z* (%)=203 (M$^+$, 100), 188 (80), 172 (80), 161 (15), 143 (10), 133 (20), 130 (15), 115 (10), 89 (10), 77 (10), 57 (5). HRMS ($C_{12}H_{13}NO_2$): Calcd 203.0946, found 203.0952.

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Parallel solid-phase synthesis of 2-arylamino-6*H*-pyrano[2,3-*f*]benzimidazole-6-ones

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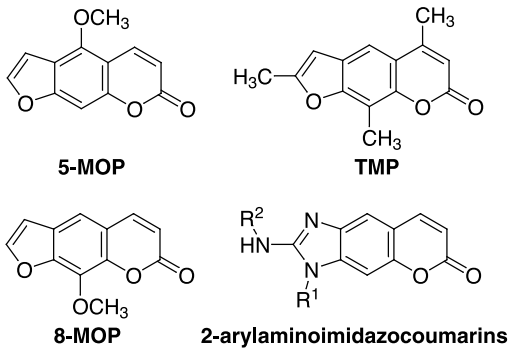
Abstract—The parallel solid-phase synthesis of a hydrophilic psoralen analogue, 2-arylamino-6*H*-pyrano[2,3-*f*]benzimidazole-6-ones (2-arylaminoimidazocoumarins), has been developed. The resin-bound 7-fluoro-4-methyl-6-nitro-2-oxo-2*H*-1-benzopyran-3-carboxylic acid underwent aromatic substitution with primary amines, followed by reduction of the nitro group with tin(II) chloride. The cyclization of the *o*-dianilino intermediates was accomplished with aryl isothiocyanates in the presence of 1,3-diisopropylcarbodiimide (DIC). The final products were released from the resin with trifluoroacetic acid (TFA) and obtained in high purity and good isolated yield. The 2-arylaminoimidazocoumarins exhibit interesting spectral properties.

1. Introduction

Coumarins (2*H*-1-benzopyran-2-ones) represent an important class of natural products displaying diverse biological activities, such as antibiotic, anticoagulant, anticancer, anti-inflammatory effects.[1] A number of natural or synthetic coumarins have extensive pharmaceutical applications.[2] In addition, coumarin derivatives also exhibit interesting spectral and optical properties. Some coumarin derivatives have been widely used as fluorescent probes,[3] active media for tunable dye lasers,[4] optical bleaching agents,[5] and triplet sensitizers.[6] Psoralens (Scheme 1), which have a linear furocoumarin structure, are well known photosensitizing drugs used in PUVA (psoralen plus UVA) photochemotherapy to treat a number of skin diseases,[7] and in photopheresis, for preventing rejection in organ transplantation[8] and for the treatment of T-cell lymphoma[9] and other autoimmune diseases.[10] The psoralens currently used in the clinic include 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP) and 4,5′,8-trimethylpsoralen (TMP) (Scheme 1). PUVA therapy has met great success in the treatment of a number of diseases, although it also causes some toxic side-effects, such as persistent erythema,[7] genotoxicity[11] and carcinogenicity,[12] as a result its direct action on DNA.[13] Work towards increasing the photoreactivity and reducing toxic effects has led to the development of a number of psoralen analogues including



Scheme 1. Chemical structures of psoralens for PUVA therapy.

pyrrolocoumarins,[14] azapsoralens,[15] furoquinolinones[16] and triazolocoumarins.[17] Their synthesis, spectral properties and biological effects have been reported. Modification of psoralens with hydrophilic side chains has also been investigated to enhance their water solubility and binding affinity to DNA.[18]

In the past few years, significant emphasis has been put on the synthesis and screening of small molecule libraries based on natural products as templates.[19] We report here a solid-phase approach for the combinatorial synthesis of a novel psoralen analogue, 2-arylamino-6*H*-pyrano[2,3-*f*]benzimidazole-6-ones (2-arylaminoimidazocoumarins, Scheme 1). It is well known that benzimidazole is an important heterocyclic nucleus in medicinal chemistry and 2-substituted benzimidazoles have shown a broad range of biological activities, such as antiviral, antiulcer and

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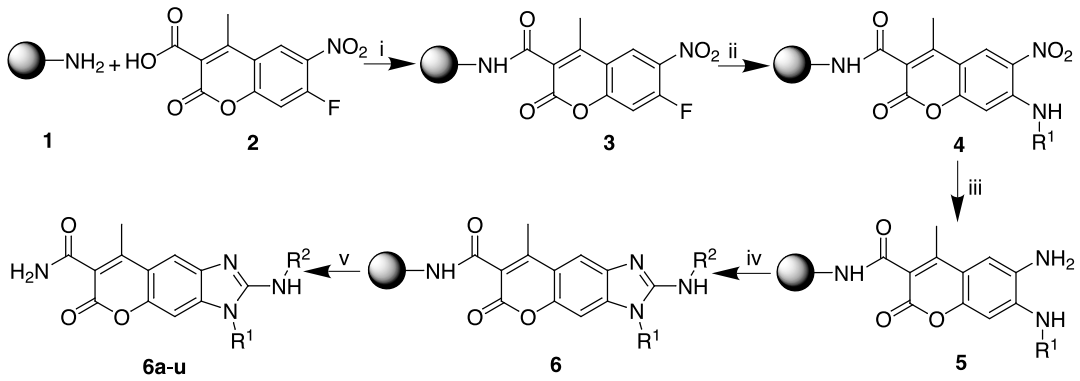


Scheme 2. Solid-phase synthesis of 2-arylamino-6*H*-pyrano[2,3-*f*]benzimidazole-6-ones. Reagents and conditions: **1**, Rink amide resin; (i) 2 equiv. of 7-fluoro-4-methyl-6-nitro-2-oxo-2*H*-1-benzopyran-3-carboxylic acid, 2 equiv. of DIC and 2 equiv. of HOBt in DMF, rt, 12 h; (ii) 2 equiv. of R^1NH_2 in 5% DIEA/DMF, rt, overnight; (iii) 2 M of $SnCl_2{\cdot}H_2O$ in DMF, rt, 20 h; (iv) 1 M of R^2NCS and 1 M of DIC in DMF, rt, overnight; (v) 95% TFA/H_2O, rt, 2 h.

antitumor effects.[20] The introduction of an imidazole moiety into a natural product scaffold such as coumarin ring to form a benzimidazole structure may confer interesting biological and physical properties in the final products. We anticipate that some of these compounds will have spectral and chemical properties that are suitable for PUVA therapy or other therapeutic applications.

2. Results and discussion

2.1. Synthesis of 2-arylamino-6*H*-pyrano[2,3-*f*]benzimidazole-6-ones

The general synthetic strategy for 2-arylamino-6*H*-pyrano[2,3-*f*]benzimidazole-6-ones **6** is shown in Scheme 2. The synthesis was started from our previously published scaffold, 7-fluoro-4-methyl-6-nitro-2-oxo-2*H*-1-benzopyran-3-carboxylic acid **2**.[21] The literature method for preparation of 2-arylaminobenzimidazoles was modified and used for the solid-phase synthesis of **6**.[22,23] The resin-bound aryl fluoride was replaced by a primary amine, followed by reduction of the nitro group. Subsequent cyclization was accomplished with isothiocyanates in the presence of 1,3-diisopropylcarbodiimide (DIC).

Scaffold **2** was ligated to Rink amide resin via its carboxyl group using DIC/1-hydroxybenzotriazole (HOBt) as the activating system. Initial attempts to carry out the aromatic nucleophilic substitution of resin-bound scaffold **3** using the literature method[22] failed to produce clean products. At a high concentration (1 M), the primary amine attacked the lactone ring resulting in complex side products, which were not separated and characterized. Thus, we tried to reduce the amine concentration to avoid the side reactions and ensure the complete substitution at the same time. The optimal reaction conditions were found to be 2 equivalents of the amine in 5% *N*,*N*-diisopropylethylamine (DIEA)/*N*,*N*-dimethylformamide (DMF). After overnight incubation at room temperature, 52 primary amines (see Table 1 for the representative structures) reacted with the resin-bound scaffold **3** to provide intermediates **4** in almost quantitative yield according to HPLC and ^{1}H NMR analysis after trifluoroacetic acid (TFA) cleavage.

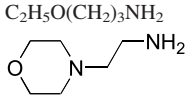
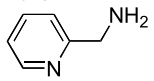
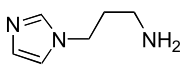
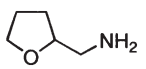
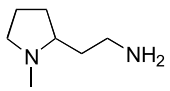
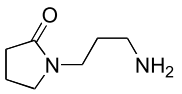
The reduction of resin-bound *o*-nitroaniline intermediates **4** was accomplished by treatment with Tin(II) chloride. The final cyclization step was studied with 21 of isothiocyanates representing a range of steric and electronic characteristics (Table 1). Aryl isothiocyanates reacted with the resin-bound *o*-dianilino intermediates **5** smoothly to afford desired products **6** after incubation at room temperature overnight in the presence of DIC. 3-Nitrophenyl isothiocyanate was an exception because it required prolonged reaction time (72 h). The cyclization was neither hindered by the substituents on 2- and 6-positions of the aryl isothiocyanate (**6b**), nor by the secondary alkyl in 7-alkylamino group of **5** (**6c**–**e**).

The intermediates involved in the DIC-catalyzed cyclization were assumed to be the highly reactive carbodiimides generated in situ from the desulfurization of the thiourea intermediates (Scheme 3).[24,25] A mechanistic study using compound **6h** as a model showed that the initial reaction with 3-nitrophenyl isothiocyanates occurred exclusively on the primary amino group to give intermediate **7h** according to ^{1}H NMR analysis. Both protons, H^a (6.49 ppm, t, J=5.9 Hz) and H^b (9.67 ppm, s), were clearly shown in ^{1}H NMR spectrum of **7h**, while no significant shift was observed for the methylene proton (3.66 ppm, m) on N-7 after the reaction.

We also examined the reactivity of alkyl isothiocyanates for the DIC-catalyzed cyclization. 2-Alkylamino-6*H*-pyrano[2,3-*f*]benzimidazole-6-ones were obtained under the same conditions, but their yield and purity were not ideal (Table 1, **6t** and **6u**), probably as a result of the low conversion efficiency of the alkyl thioureas to carbodiimides.[25,26]

Using this solid-phase approach, a small library containing 19 2-arylamino-6*H*-pyrano[2,3-*f*]benzimidazole-6-ones (**6a**–**s**) and 2 2-alkylamino-6*H*-pyrano[2,3-*f*]benzimidazole-6-ones (**6t** and **6u**) was prepared. All of the 2-arylamino-6*H*-pyrano[2,3-*f*]benzimidazole-6-ones were obtained in high purity (83–97%) and good isolated yield (67–83%). The library compounds have two points of diversity, and an additional diversity point could be introduced to the carboxyl group via coupling an amino acid prior to the scaffold attachment.

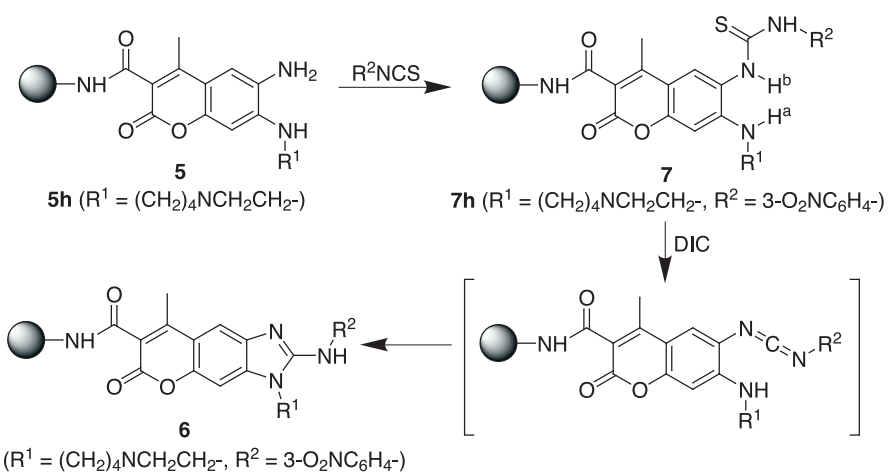
Table 1. Synthesis and spectral properties of compounds **6a**–**u** produced via Scheme 2[a]

Entry	R^1NH_2	R^2NCS	Yield %	Purity %	λ_{abs} nm	λ_{em} nm	Φ
6a	$CH_3(CH_2)_3NH_2$	C_6H_5NCS	80	91	353	509	0.22
6b	$CH_3(CH_2)_2NH_2$	2,6-$(CH_3)_2C_6H_3NCS$	71	87	341	501	0.21
6c	$C_2H_5(CH_3)CHNH_2$	4-FC_6H_4NCS	76	92	358	509	0.10
6d	*c*-$C_5H_9NH_2$	4-ClC_6H_4NCS	76	91	363	507	0.18
6e	*c*-$C_6H_{11}NH_2$	4-BrC_6H_4NCS	72	84	363	508	0.21
6f	$C_6H_5CH_2NH_2$	4-$CH_3OC_6H_4NCS$	83	96	351	—[b]	0
6g	4-$ClC_6H_4CH_2CH_2NH_2$	3-$CH_3OC_6H_4NCS$	71	85	354	511	0.03
6h	$(CH_2)_4NCH_2CH_2NH_2$	3-$O_2NC_6H_4NCS$[c]	70	83	369	—[b]	0
6i	$C_2H_5O(CH_2)_3NH_2$	4-$O_2NC_6H_4NCS$	72	88	382	—[b]	0
6j		1-$C_{10}H_7NCS$	71	86	349	—[b]	0
6k	2,4-$(CH_3O)_2C_6H_3CH_2NH_2$	4-IC_6H_4NCS	80	96	362	509	0.16
6l	$C_6H_5OCH_2CH_2NH_2$	3-BrC_6H_4NCS	79	97	363	498	0.44
6m		3-ClC_6H_4NCS	82	96	360	495	0.03
6n		3-FC_6H_4NCS	72	92	365	492	0.48
6o		2-FC_6H_4NCS	81	97	362	492	0.48
6p		2-ClC_6H_4NCS	79	94	373	493	0.07
6q	4-$CH_3OC_6H_4CH_2CH_2NH_2$	3,5-$Cl_2C_6H_3NCS$	70	86	363	492	0.40
6r		3,4-$Cl_2C_6H_3NCS$	67	89	364	501	0.43
6s	$(CH_2)_5NCH_2CH_2NH_2$	2,4-$Cl_2C_6H_3NCS$	69	88	367	476	0.02
6t	$(CH_3)_2CHCH_2NH_2$	$CH_3(CH_2)_2NCS$	37	41	344	515	0.19
6u	3,4-$(CH_2O_2)C_6H_3CH_2NH2$	$CH_3(CH_2)_3NCS$	45	59	355	512	0.22

[a] Yields were calculated based on the purified products by preparative HPLC with purity >95%. Purity was determined by HPLC analysis (UV detection at 220 nm) of crude products. λ_{abs} and λ_{em} represent the maximum absorption and fluorescence wavelength, respectively. Φ is the fluorescence quantum yield of compounds in ethanol, and was determined using 7-amino-4-methylcoumarin as the standard reference.
[b] No detectable fluorescence under the experimental conditions.
[c] The cyclization was allowed to proceed for 72 h.

2.2. Spectral properties of library compounds

The spectral properties of compounds **6a**–**u** are summarized in Table 1. The absorption and fluorescence spectra of compound **6l** are shown in Figure 1 as an example. All of the 21 compounds have intense absorption in the range of 320–390 nm. Their absorption spectra match well with the output of the UVA lamp for PUVA therapy (maximum emission at 365 nm).[27] When compared to the psoralen sensitizers (maximum absorption around 300 nm) currently used in the



Scheme 3. Mechanistic study of DIC-catalyzed cyclization.

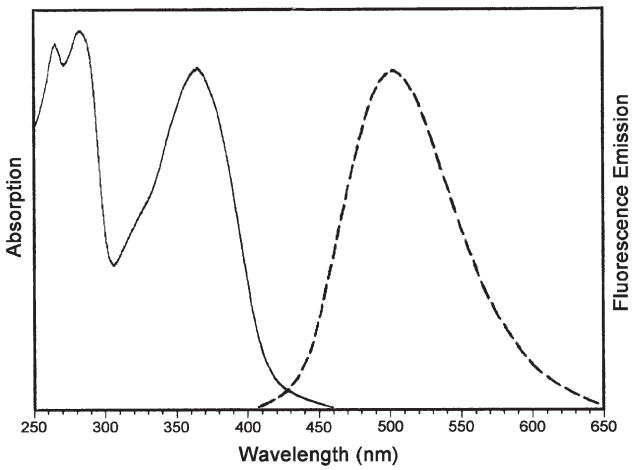


Figure 1. Normalized absorption (solid line) and fluorescence (dashed line) spectra of compound **6l**.

clinic, 2-arylamino-6*H*-pyrano[2,3-*f*]benzimidazole-6-ones absorb UVA much more efficiently.

Many of the library compounds exhibit green or yellow-green fluorescence between 450 and 600 nm. Since R^2 is conjugated to the imidazocoumarin ring via a nitrogen, the substituents in the aryl have significant effects on the spectral properties of 2-arylamino-6*H*-pyrano[2,3-*f*]benzimidazole-6-ones. When the aryl bears a strong electron-donating or electron-withdrawing group, the compound gives very weak fluorescence (**6f–i**). Among the 21 library compounds, **6l**, **6n**, **6o**, **6q** and **6r** exhibit stronger fluorescence than others. Furthermore, all of the fluorescent compounds have a large Stokes shift (120–170 nm). The overlap between their absorption and fluorescence spectra is very small. Very few low molecular weight dyes have a combination of a large Stokes shift and high fluorescence output,[28] therefore this unique property gives 2-arylamino-6*H*-pyrano[2,3-*f*]benzimidazole-6-ones a great potential for use in multicolor fluorescent labeling applications.

3. Conclusion

A parallel solid-phase method for the preparation of 2-arylamino-6*H*-pyrano[2,3-*f*]benzimidazole-6-ones has been developed. The desired products were obtained in high purity with good yield after five reaction steps. Some of the synthesized compounds exhibit promising spectral properties. A third point of diversity can be introduced to 2-arylamino-6*H*-pyrano[2,3-*f*]benzimidazole-6-ones by attaching the first building block to the solid support prior to the synthesis of the compound. The guanidino group in the 2-aminoimidazole structure of 2-arylamino-6*H*-pyrano[2,3-*f*]benzimidazole-6-ones is expected to improve the water solubility of these compounds. The solid-phase synthesis approach described in this report is ideal for automated applications because all the reactions were performed under mild conditions. We anticipate that fluorescent probes and photosensitizers for PUVA therapy with desirable spectral properties can be readily discovered by synthesizing and screening a diverse 'one-bead one-compound' library of 2-arylamino-6*H*-pyrano[2,3-*f*] benzimidazole-6-ones, encoded by our recently described encoding method for small molecule libraries.[29]

4. Experimental

4.1. General

7-Fluoro-4-methyl-6-nitro-2-oxo-2*H*-1-benzopyran-3-carboxylic acid was prepared using our published procedure.[21] DIC and TFA were purchased from Advanced ChemTech (Louisville, KY). Rink amide MBHA resin (0.45 mmol/g) and HOBt were purchased from GL Biochem (Shanghai, China). All solvents and other chemical reagents were purchased from Aldrich (Milwaukee, WI) and were analytical grade. Analytical HPLC analyses (Vydac column, 4.6 mm×250 mm, 5 μm, 300 Å, C_{18}, 1.0 mL/min, 25 min gradient from 100% aqueous media (0.1% TFA) to 100% CH_3CN (0.1% TFA), 214, 220, 254 and 280 nm) and preparative HPLC purification (Vydac column, 20 mm×250 mm, 5 μm, 300 Å, C_{18}, 7.0 mL/min, 45 min gradient from 100% aqueous media (0.1% TFA) to 100% CH_3CN (0.1% TFA), 254 nm) were performed on a Beckman System Gold HPLC system (Fullerton, CA). UV–Vis absorption spectra were recorded on a Hewlett–Packard 8425A diode array spectrophotometer (Palo Alto, CA) using 5×10^{-5} mol/L solutions in ethanol. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer (Palo Alto, CA). Samples were excited at their maximum absorption wavelength. Diluted solutions in ethanol (2.5×10^{-7} mol/L) were used to minimize self-quenching. Fluorescence quantum yields were measured using the literature method based on a value of 0.88 for 7-aminocoumarin.[30] ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX 500 MHz spectrometer (Billerica, MA) at 25 °C. All of the experiments are carried out at room temperature unless otherwise noted.

4.2. General procedure for solid-phase synthesis of 2-arylamino-6*H*-pyrano[2,3-*f*]benzimidazole-6-ones

Rink amide MBHA resin (100 mg, 0.045 mmol) was swollen in DMF overnight. The supernatant was removed, and a 20% piperidine solution in DMF (1 mL) was added to the resin. The mixture was shaken for 15 min, and the supernatant was removed. This process was repeated. The resin was washed with DMF, methanol (MeOH), and DMF. To the resin was added a solution of 7-fluoro-4-methyl-6-nitro-2-oxo-2*H*-1-benzopyran-3-carboxylic acid (24.1 mg, 0.090 mmol), HOBt (12.2 mg, 0.090 mmol) and DIC (14.1 μL, 0.090 mmol) in DMF (1 mL). The resulting mixture was shaken for 12 h. The complete coupling was confirmed by a negative ninhydrin test.[31] The supernatant was removed, and the resin was washed with DMF, dichloromethane (DCM), MeOH, and DMF. To the resin was added a solution of a primary amine (0.090 mmol) in DMF (2 mL). The resulting mixture was shaken overnight. The supernatant was removed, and the resin was washed with DMF, DCM, MeOH, and DMF. To the resin was added 2 M $SnCl_2{\cdot}H_2O$ solution in DMF (2 mL), and the resulting mixture was shaken for 20 h. The supernatant was removed, and the resin was washed with DMF, DCM, MeOH, and

DMF. To the resin was added 1 M isothiocyanate and 1 M DIC solution in DMF (2 mL). The resulting mixture was shaken overnight. The supernatant was removed. The resin was washed with DMF, DCM, MeOH, and DCM, and then dried in vacuo. To the dried resin was added 2 mL of 95% TFA solution in water at ice-bath temperature. The mixture was slowly warmed to room temperature and allowed to mix for 2 h. The supernatant was then removed and the resin was washed with neat TFA (3×1 mL). The combined supernatants were concentrated to dryness under a stream of nitrogen, and further dried in vacuo. The crude products were analyzed and purified by HPLC.

4.2.1. 3-Butyl-8-methyl-6-oxo-2-(phenylamino)-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6a. Yellow solid; yield 80%; ^{1}H NMR (DMSO-d_6) δ 10.1 (s, br, 1H), 7.86 (s, 1H), 7.72 (d, 2H, J=7.9 Hz), 7.70 (s, 1H), 7.67 (s, 1H), 7.64 (s, 1H), 7.46 (t, 2H, J=7.9 Hz), 7.22 (t, 1H, J=7.4 Hz), 4.32 (t, 2H, J=7.3 Hz), 2.45 (s, 3H), 1.74 (m, 2H), 1.37 (m, 2H), 0.92 (t, 3H, J=7.4 Hz); ^{13}C NMR (DMSO-d_6) δ 166.7, 158.9, 151.5, 149.3, 149.0, 138.7, 136.2, 133.4, 130.0, 125.3, 122.9, 122.3, 115.1, 110.4, 97.9, 43.2, 30.9, 20.0, 16.8, 14.4; ESI-MS m/z 391.2 (MH$^+$).

4.2.2. 2-[(2,6-Dimethylphenyl)amino]-8-methyl-6-oxo-3-propyl-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6b. Yellow solid; yield 71%; ^{1}H NMR (DMSO-d_6) δ 10.8 (s, br, 1H), 7.90 (s, 1H), 7.89 (s, 1H), 7.66 (s, 1H), 7.53 (s, 1H), 7.38–7.29 (m, 4H), 4.36 (t, 2H, J=7.2 Hz), 2.40 (s, 3H), 2.25 (s, 6H), 1.88 (m, 2H), 1.01 (t, 3H, J=7.3 Hz); ^{13}C NMR (DMSO-d_6) δ 166.4, 158.5, 150.8, 150.0, 148.3, 137.1, 135.1, 132.9, 129.8, 129.7, 127.9, 123.8, 115.8, 108.5, 99.2, 45.1, 21.8, 18.3, 16.6, 11.3; ESI-MS m/z 405.2 (MH$^+$).

4.2.3. 2-[(4-Fluorophenyl)amino]-8-methyl-3-(1-methylpropyl)-6-oxo-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6c. Yellow solid; yield 76%; ^{1}H NMR (DMSO-d_6) δ 9.9 (s, br, 1H), 7.85 (s, 1H), 7.77–7.70 (m, 4H), 7.64 (s, 1H), 7.29 (t, 2H, J=9.0 Hz), 4.72 (m, 1H), 2.44 (s, 3H), 2.14 (m, 1H), 1.97 (m, 1H), 1.63 (d, 3H, J=6.8 Hz), 0.80 (t, 3H, J=7.3 Hz); ^{13}C NMR (DMSO-d_6) δ 166.7, 159.6 (d, $^1J_{CF}$=237.6 Hz), 158.9, 152.1, 148.9, 148.8, 135.5, 134.3, 124.2 (d, $^3J_{CF}$=7.9 Hz), 123.0, 117.8, 116.6 (d, $^2J_{CF}$=22.3 Hz), 114.9, 110.7, 99.6, 53.5, 27.0, 18.7, 16.7, 11.4; ESI-MS m/z 409.2 (MH$^+$).

4.2.4. 2-[(4-Chlorophenyl)amino]-3-cyclopentyl-8-methyl-6-oxo-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6d. Yellow solid; yield 76%; ^{1}H NMR (DMSO-d_6) δ 9.9 (s, br, 1H), 7.87 (s, 1H), 7.81–7.75 (m, 3H), 7.64 (s, 1H), 7.49–7.44 (m, 3H), 5.07 (m, 1H), 2.46 (s, 3H), 2.22–2.00 (m, 6H), 1.73 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 166.8, 159.0, 151.8, 149.0, 148.5, 138.8, 136.3, 134.0, 129.6, 127.8, 122.8, 122.7, 114.8, 111.4, 99.0, 55.9, 29.1, 25.0, 16.7; ESI-MS m/z 437.1 (MH$^+$).

4.2.5. 2-[(4-Bromophenyl)amino]-3-cyclohexyl-8-methyl-6-oxo-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6e. Yellow solid; yield 72%; ^{1}H NMR (DMSO-d_6) δ 9.9 (s, br, 1H), 7.87 (s, 1H), 7.81 (s, 1H), 7.74 (s, 1H), 7.72 (d, 2H, J=8.7 Hz), 7.63 (s, 1H), 7.60 (d, 2H, J=8.7 Hz), 4.55 (t, 1H, J=12.0 Hz), 2.45 (s, 3H), 2.24 (m, 2H), 1.90 (m, 4H), 1.67 (m, 1H), 1.49 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 166.8, 159.0, 151.2, 149.0, 148.6, 139.2, 135.9, 134.7, 132.6, 123.4, 122.8, 116.0, 114.7, 111.1, 99.9, 55.2, 30.0, 26.1, 24.8, 16.7; ESI-MS m/z 495.1 (MH$^+$).

4.2.6. 3-Benzyl-2-[(4-methoxyphenyl)amino]-8-methyl-6-oxo-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6f. Yellow solid; yield 83%; ^{1}H NMR (DMSO-d_6) δ 10.3 (s, br, 1H), 7.83 (s, 1H), 7.68 (s, 1H), 7.64 (s, 1H), 7.61 (d, 2H, J=8.8 Hz), 7.41–7.29 (m, 5H), 7.05 (d, 2H, J=8.8 Hz), 5.61 (s, 2H), 3.79 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 166.6, 158.7, 157.6, 152.3, 149.3, 148.8, 136.0, 135.9, 133.0, 130.9, 129.6, 128.6, 127.8, 124.7, 124.6, 123.1, 115.4, 110.1, 98.2, 56.1, 46.4, 16.8; ESI-MS m/z 455.2 (MH$^+$).

4.2.7. 3-[2-(4-Chlorophenyl)ethyl]-2-[(3-methoxyphenyl)amino]-8-methyl-6-oxo-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6g. Yellow solid; yield 71%; ^{1}H NMR (DMSO-d_6) δ 10.2 (s, br, 1H), 7.87 (s, 1H), 7.66 (m, 2H), 7.60 (s, 1H), 7.36 (t, 1H, J=8.1 Hz), 7.33–7.25 (m, 4H), 7.21 (s, 1H), 7.13 (d, 1H, J=7.2 Hz), 6.82 (d, 1H, J=7.2 Hz), 4.58 (t, 2H, J=6.7 Hz), 3.80 (s, 3H), 3.08 (t, 2H, J=6.7 Hz), 2.44 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 166.7, 160.7, 158.8, 151.3, 149.4, 148.8, 139.4, 137.2, 135.7, 132.1, 131.8, 130.8, 128.9, 123.2, 123.1, 115.3, 114.7, 111.2, 110.2, 108.3, 98.3, 56.0, 44.3, 33.6, 16.8; ESI-MS m/z 503.2 (MH$^+$).

4.2.8. 8-Methyl-2-[(3-nitrophenyl)amino]-6-oxo-3-[2-(1-pyrrolidinyl)ethyl]-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6h. Yellow solid; yield 70%; ^{1}H NMR (DMSO-d_6) δ 10.1 (s, br, 1H), 8.87 (s, 1H), 8.25 (s, 1H), 7.91–7.83 (m, 3H), 7.67 (t, 1H, J=8.2 Hz), 7.64 (m, 2H), 4.70 (t, 2H, J=6.2 Hz), 3.65 (t, 2H, J=6.2 Hz), 3.63–3.44 (m, 2H), 3.30–3.11 (m, 2H), 2.50 (s, 3H), 2.10–1.82 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 166.9, 159.0, 151.5, 149.5, 149.0, 148.9, 142.5, 137.0, 130.8, 125.5, 122.4, 118.2, 117.1, 114.8, 113.6, 112.5, 97.2, 54.6, 52.0, 39.4, 23.5, 16.9; ESI-MS m/z 477.2 (MH$^+$).

4.2.9. 3-(3-Ethoxypropyl)-8-methyl-2-[(4-nitrophenyl)amino]-6-oxo-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6i. Yellow solid; yield 72%; ^{1}H NMR (DMSO-d_6) δ 9.9 (s, br, 1H), 8.28 (d, 2H, J=9.2 Hz), 8.13 (d, 2H, J=9.2 Hz), 7.90 (s, 1H), 7.85 (s, 1H), 7.62 (s, 1H), 7.50 (s, 1H), 4.42 (t, 2H, J=6.2 Hz), 3.27 (m, 4H), 2.49 (s, 3H), 1.98 (m, 2H), 1.04 (t, 3H, J=7.0 Hz); ^{13}C NMR (DMSO-d_6) δ 166.9, 159.1, 151.0, 149.5, 149.0, 147.3, 141.6, 138.6, 137.4, 125.8, 122.4, 118.5, 114.8, 113.1, 97.2, 67.0, 66.1, 40.4, 29.2, 16.9, 15.6; ESI-MS m/z 466.2 (MH$^+$).

4.2.10. 8-Methyl-3-[2-(4-morpholinyl)ethyl]-2-(1-naphthalenylamino)-6-oxo-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6j. Yellow solid; yield 71%; ^{1}H NMR (DMSO-d_6) δ 9.9 (s, br, 1H), 8.19 (d, 1H, J=7.9 Hz), 8.13 (d, 1H, J=7.7 Hz), 7.94 (d, 1H, J=7.7 Hz), 7.89 (s, 1H), 7.73 (s, 1H), 7.68–7.54 (m, 5H), 7.42 (s, 1H), 4.78 (t, 2H, J=6.1 Hz), 3.85 (m, 4H), 3.70 (t, 2H, J=6.1 Hz), 3.47 (m, 4H), 2.37 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 166.6, 158.7, 152.2, 149.6, 148.7, 135.8, 135.0, 130.7, 129.7, 128.9, 127.3, 127.0, 123.9, 123.0, 121.2, 120.4, 118.1, 115.7, 115.0, 107.7, 97.9, 64.3, 53.9, 52.4, 38.3, 16.7; ESI-MS m/z 498.2 (MH$^+$).

4.2.11. 3-[(2,4-Dimethoxyphenyl)methyl]-2-[(4-iodophenyl)amino]-8-methyl-6-oxo-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6k. Yellow solid; yield 80%; ^{1}H NMR (DMSO-d_6) δ 10.0 (s, br, 1H), 7.81 (s, 1H), 7.79–7.73 (m, 3H), 7.65 (d, 2H, J=8.3 Hz), 7.62 (s, 1H), 7.31 (s, 1H), 7.10 (d, 1H, J=8.0 Hz), 6.58 (s, 1H), 6.48 (d, 1H, J=8.0 Hz), 5.44 (s, 2H), 3.76 (s, 3H), 3.72 (s, 3H), 2.44 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 166.8, 161.4, 158.9, 158.7, 151.8, 149.1, 148.9, 139.5, 138.5, 136.4, 135.8, 130.1, 123.1, 122.7, 115.6, 115.0, 111.2, 105.4, 99.4, 98.0, 87.7, 56.3, 56.0, 42.7, 16.8; ESI-MS m/z 611.1 (MH^+).

4.2.12. 2-[(3-Bromophenyl)amino]-8-methyl-6-oxo-3-(2-phenoxyethyl)-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6l. Yellow solid; yield 79%; ^{1}H NMR (DMSO-d_6) δ 9.8 (s, br, 1H), 8.21 (s, 1H), 7.86 (s, 1H), 7.80 (s, 1H), 7.78 (d, 1H, J=8.2 Hz), 7.62 (s, 1H), 7.61 (s, 1H), 7.37 (t, 1H, J=8.0 Hz), 7.28 (m, 1H), 7.23 (t, 2H, J=7.6 Hz), 6.89 (t, 1H, J=7.3 Hz), 6.79 (d, 2H, J=8.5 Hz), 4.76 (t, 2H, J=4.8 Hz), 4.35 (t, 2H, J=4.8 Hz), 2.47 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 166.9, 159.1, 158.6, 151.9, 149.4, 149.0, 141.7, 137.3, 137.0, 131.6, 130.3, 126.1, 122.5, 121.7, 121.6, 119.2, 115.2, 115.0, 114.9, 111.8, 97.9, 66.4, 42.9, 16.9; ESI-MS m/z 533.1 (MH^+).

4.2.13. 2-[(3-Chlorophenyl)amino]-8-methyl-6-oxo-3-(2-pyridinylmethyl)-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6m. Yellow solid; yield 82%; ^{1}H NMR (DMSO-d_6) δ 10.3 (s, br, 1H), 8.54 (d, 1H, J=4.1 Hz), 8.09 (s, 1H), 7.90–7.84 (m, 2H), 7.83 (s, 1H), 7.66 (d, 1H, J=8.2 Hz), 7.62 (s, 1H), 7.47 (s, 1H), 7.44–7.39 (m, 2H), 7.36 (t, 1H, J=6.0 Hz), 7.15 (d, 1H, J=7.9 Hz), 5.74 (s, 2H), 2.48 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 166.8, 159.0, 155.2, 152.2, 149.8, 149.3, 149.1, 141.4, 138.6, 137.1, 136.9, 134.1, 131.3, 124.0, 123.3, 122.6, 122.5, 119.6, 118.8, 115.0, 112.0, 97.6, 47.6, 16.9; ESI-MS m/z 460.1 (MH^+).

4.2.14. 2-[(3-Fluorophenyl)amino]-3-[3-(1*H*-imidazol-1-yl)propyl]-8-methyl-6-oxo-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6n. Yellow solid; yield 72%; ^{1}H NMR (DMSO-d_6) δ 9.7 (s, br, 1H), 9.09 (s, 1H), 7.96 (d, 1H, J=7.0 Hz), 7.86 (s, 1H), 7.85 (s, 1H), 7.78 (s, 1H), 7.67 (s, 1H), 7.62 (s, 1H), 7.57 (d, 1H, J=8.1 Hz), 7.55 (s, 1H), 7.40 (m, 1H), 6.88 (t, 1H, J=8.0 Hz), 4.43 (t, 2H, J=6.6 Hz), 4.32 (t, 2H, J=7.2 Hz), 2.48 (s, 3H), 2.37 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 166.9, 163.1 (d, $^1J_{CF}$=239.3 Hz), 159.1, 151.7, 149.5, 148.9, 142.3 (d, $^3J_{CF}$=9.9 Hz), 138.2, 137.1, 136.1, 131.0 (d, $^3J_{CF}$=9.5 Hz), 122.5, 122.3, 120.7, 115.8, 114.7, 112.2, 109.4 (d, $^2J_{CF}$=20.4 Hz), 106.7 (d, $^2J_{CF}$=26.9 Hz), 97.0, 47.1, 40.3, 29.4, 16.3; ESI-MS m/z 461.2 (MH^+).

4.2.15. 2-[(2-Fluorophenyl)amino]-8-methyl-6-oxo-3-[(tetrahydro-2-furanyl)methyl]-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6o. Yellow solid; yield 81%; ^{1}H NMR (DMSO-d_6) δ 9.8 (s, br, 1H), 8.06 (t, 1H, J=7.6 Hz), 7.85 (s, 1H), 7.74 (s, 1H), 7.68 (s, 1H), 7.63 (s, 1H), 7.37 (t, 1H, J=9.6 Hz), 7.30 (t, 1H, J=7.6 Hz), 7.24 (m, 1H), 4.48 (d, 1H, J=15.1 Hz), 4.32 (dd, 1H, J=15.1, 7.3 Hz), 4.31 (m, 1H), 3.86 (m, 1H), 3.73 (m, 1H), 2.45 (s, 3H), 2.11 (m, 1H), 1.86 (m, 2H), 1.62 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 166.8, 158.9, 154.8 (d, $^1J_{CF}$=243.1 Hz), 152.2, 149.2, 149.1, 137.3, 127.0 (d, $^2J_{CF}$=11.3 Hz), 126.2, 125.7, 124.3, 122.8, 116.6 (d, $^2J_{CF}$=13.6 Hz), 115.1, 111.0, 98.2, 47.7, 47.4, 44.8, 28.9, 26.1, 16.8; ESI-MS m/z 437.2 (MH^+).

4.2.16. 2-[(2-Chlorophenyl)amino]-8-methyl-3-[2-(1-methyl-2-pyrrolidinyl)ethyl]-6-oxo-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6p. Yellow solid; yield 79%; ^{1}H NMR (DMSO-d_6) δ 10.0 (s, br, 1H), 7.86 (s, 1H), 7.67 (s, 1H), 7.65–7.57 (m, 3H), 7.56 (s, 1H), 7.44 (t, 1H, J=6.9 Hz), 7.29 (t, 1H, J=6.9 Hz), 4.35 (t, 2H, J=6.8 Hz), 3.59 (m, 1H), 3.37 (m, 1H), 3.08 (m, 1H), 2.84 (s, 3H), 2.44 (m, 1H), 2.41 (s, 3H), 2.26 (m, 1H), 2.09–1.97 (m, 2H), 1.91 (m, 1H), 1.80 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 166.8, 158.9, 151.4, 149.3, 149.0, 136.4, 130.7, 129.2, 128.9, 127.3, 122.6, 118.1, 115.8, 114.7, 113.4, 109.0, 97.4, 66.5, 55.9, 40.9, 39.6, 29.7, 29.2, 22.0, 16.8; ESI-MS m/z 480.2 (MH^+).

4.2.17. 2-[(3,5-Dichlorophenyl)amino]-3-[2-(4-methoxyphenyl)ethyl]-8-methyl-6-oxo-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6q. Yellow solid; yield 70%; ^{1}H NMR (DMSO-d_6) δ 9.6 (s, br, 1H), 7.85 (s, 2H), 7.81 (s, 2H), 7.62 (s, 1H), 7.46 (s, 1H), 7.21 (s, 1H), 7.03 (d, 2H, J=8.4 Hz), 6.71 (d, 2H, J=8.4 Hz), 4.53 (t, 2H, J=6.3 Hz), 3.61 (s, 1H), 2.95 (t, 2H, J=6.3 Hz), 2.45 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 166.9, 159.1, 158.7, 151.3, 149.5, 149.0, 142.8, 137.5, 136.8, 134.7, 130.7, 130.1, 122.4, 121.9, 117.7, 114.7, 114.4, 112.5, 97.5, 55.6, 44.5, 33.9, 16.9; ESI-MS m/z 537.1 (MH^+).

4.2.18. 2-[(3,4-Dichlorophenyl)amino]-8-methyl-6-oxo-3-[3-(2-oxo-1-pyrrolidinyl)propyl]-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6r. Yellow solid; yield 67%; ^{1}H NMR (DMSO-d_6) δ 9.9 (s, br, 1H), 8.30 (d, 1H, J=1.9 Hz), 7.87–7.81 (m, 2H), 7.80–7.75 (m, 1H), 7.67–7.57 (m, 3H), 4.28 (t, 2H, J=6.1 Hz), 3.37–3.25 (m, 4H), 2.49 (s, 3H), 2.22 (m, 2H), 1.93 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 175.1, 166.9, 159.0, 151.5, 149.4, 149.0, 140.3, 137.1, 136.8, 131.9, 131.4, 124.9, 122.5, 121.5, 120.5, 114.9, 112.0, 97.4, 47.1, 41.2, 40.0, 31.2, 26.9, 18.2, 16.9; ESI-MS m/z 528.1 (MH^+).

4.2.19. 2-[(2,4-Dichlorophenyl)amino]-8-methyl-6-oxo-3-[2-(1-piperidinyl)ethyl]-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6s. Yellow solid; yield 69%; ^{1}H NMR (DMSO-d_6) δ 9.6 (s, br, 1H), 7.86 (s, 1H), 7.64 (s, 1H), 7.62 (s, 1H), 7.50 (s, 1H), 7.44–7.36 (m, 2H), 7.32 (s, 1H), 4.51 (t, 2H, J=5.9 Hz), 3.54 (t, 2H, J=5.9 Hz), 3.52–3.03 (m, 4H), 2.39 (s, 3H), 1.72 (m, 4H), 1.54 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 166.8, 158.9, 150.6, 149.5, 149.0, 136.2, 129.9, 128.9, 128.8, 127.9, 126.6, 122.3, 117.9, 115.5, 114.0, 106.1, 96.9, 54.1, 53.3, 37.4, 23.4, 21.9, 16.7; ESI-MS m/z 514.1 (MH^+).

4.2.20. 8-Methyl-3-(2-methylpropyl)-6-oxo-2-(propylamino)-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6t. Yellow solid; yield 37%; ^{1}H NMR (DMSO-d_6) δ 8.9 (s, br, 1H), 7.85 (s, 1H), 7.76 (s, 1H), 7.67 (s, 1H), 7.65 (s, 1H), 3.99 (d, 2H, J=7.7 Hz), 2.46 (s, 3H), 2.15 (m, 1H), 1.67 (m, 2H), 0.95 (t, 3H, J=7.3 Hz), 0.91 (d, 6H, J=6.6 Hz); ^{13}C NMR (DMSO-d_6) δ 166.5, 158.6, 152.8, 149.7, 148.5, 139.1, 135.7, 123.4, 115.4, 108.2, 98.9, 49.6, 45.5, 27.8, 22.5, 19.9, 16.8, 11.8; ESI-MS m/z 357.2 (MH^+).

4.2.21. 3-(1,3-Benzodioxol-5-ylmethyl)-2-(butylamino)-8-methyl-6-oxo-6*H*-pyrano[2,3-*f*]benzimidazole-7-carboxamide 6u. Yellow solid; yield 45%; ^{1}H NMR (DMSO-d_6) δ 9.0 (s, br, 1H), 7.84 (s, 1H), 7.68 (s, 1H), 7.66 (s, 1H), 7.58 (s, 1H), 6.94 (s, 1H), 6.90 (d, 1H, J=8.9 Hz), 6.84 (d, 1H, J=8.9 Hz), 5.34 (s, 2H), 3.48 (m, 2H), 2.45 (s, 3H), 1.64 (m, 2H), 1.36 (m, 2H), 0.92 (t, 3H, J=7.4 Hz); ^{13}C NMR (DMSO-d_6) δ 166.5, 158.6, 152.8, 149.6, 148.6, 148.4, 147.8, 138.4, 135.2, 128.9, 123.4, 121.6, 115.5, 109.2, 108.6, 108.5, 102.0, 98.7, 46.0, 43.7, 31.1, 20.0, 16.8, 14.3; ESI-MS m/z 449.2 (MH^+).

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Combinatorial synthesis of amino acid- and peptide-carbohydrate conjugates on solid phase

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Abstract—Carbohydrates are useful polyfunctional scaffold molecules which allow the selective attachment of a number of different side chains. The combinatorial solid phase synthesis of diverse amino acid or peptide conjugates of a polyfunctional glucose scaffold based on a set of selectively removable and orthogonally stable protecting groups is described. The resulting carbohydrate-peptide hybrids constitute potential turn mimetics.

1. Introduction

As a rule, the specific interaction of peptides with their receptors involves the recognition of a short amino acid sequence adopting a characteristic secondary structure. Common binding motifs are the so called tight turns which can be classified according to the number of amino acid residues involved and the dihedral angles in the peptide backbone.[1] Considerable effort has been put in the development of new scaffold molecules which partially or completely mimic a tight turn.[2] A well known example is the construction of a mimetic of the turn found in the peptide hormone somatostatin from D-glucose.[3] Due to their polyfunctionality and conformational rigidity, monosaccharides are attractive chiral scaffolds for the preparation of peptidomimetics and other bioactive compounds.[4] Moreover, size and curvature of the hydroxy-substituted pyranose ring are comparable to the corresponding parameters of the peptide backbone found in a β-turn, the most common of the tight turns. In order to achieve selective reactions on the hydroxy functions of the scaffold, a set of selectively removable protecting groups has to be used, and the deprotection/derivatization sequences on the template should preferentially be carried out on solid phase, if repeated separation of compound mixtures is to be avoided. Recently, we have demonstrated that D-glucose can be used as a versatile scaffold for solid-phase combinatorial chemistry which allows the selective attachment of up to five different side chains via the formation of ethers, carbamates or esters as well as O-, N- or S-glycosides.[5,6] Herein, we report on methods for attaching amino acids or short peptides to one or two positions of the monosaccharide scaffold in order to incorporate the substituted pyranose ring as a potential turn mimetic into a peptide chain. These methods include the attachment via the N- as well as the C-termini, the parallel functionalization of two hydroxy groups with identical side chains and the sequential attachment or elongation of two different peptide chains. We chose scaffold **1** for the development of the synthetic methods since two typical hydroxy functions (i.e. the 6- and the 4-position) are available for selective manipulations and none of the alkyl groups in positions 2 and 3 has to be removed before activation of the thioglycoside anchor.[7] This releasing transglycosylation reaction is performed either by addition of bromine or N-bromosuccinimide (NBS) and 2,6-di-*tert*-butylpyridine (DTBP) and conversion of the generated glycosyl bromide to a glycoside by reaction with an alcohol.[5,6,8] By placing an alkyl substituent in the 2-position, a high reactivity of the intermediate glycosyl bromides is ensured, albeit at the expense of neighboring group assistance in the last substitution step at the anomeric center. Thus, the α-anomers of the resulting O-glycosides are predominantly formed. The experiments described here have also been carried out in order to explore carbohydrates as polyvalent templates for the spatial alignment of peptide chains as required for the construction of artificial receptors.

2. Results and discussion

Starting from scaffold **1**, a method had to be found to attach the N-terminus of an α-amino acid to the hydroxy group in 6-position. Since the formation of carbamates from free

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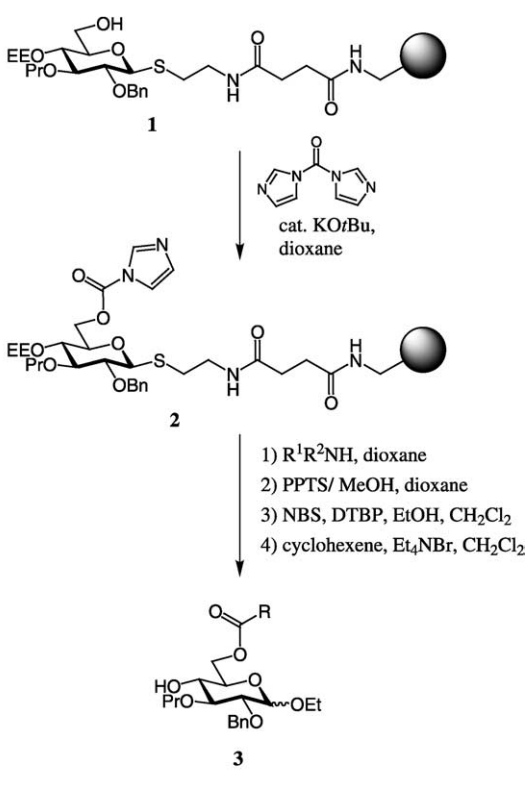
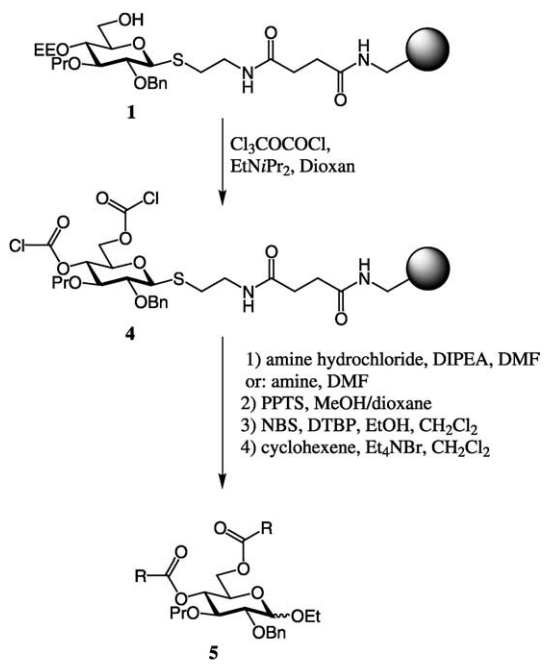
hydroxy functions with aryl or alkyl isocyanates turned out to be a very reliable derivatization reaction, one possible option would be the use of isocyanates derived from amino acid esters. As an alternative, the hydroxy group can be converted into an activated carbonate, which can be reacted with an amino acid ester or any primary or secondary amine. As the second method is more versatile and less laborious, the reaction of **1** with phosgene equivalents was investigated. The Staab reagent, 1,1′-carbonyldiimidazole (CDI), is a stable phosgene substitute of low toxicity.[9] Its reaction with **1** is slow but the reaction rate can be substantially increased by addition of catalytic amounts of bases such as potassium *tert*-butanolate. The resulting polymer-bound imidazolide **2** was treated with different primary and secondary amines to furnish the corresponding carbamates **3**. The 1-ethoxyethyl (EE) group was removed by a mild transacetalization using pyridinium-*p*-toluenesulfonate (PPTS) in a mixture of methanol and dioxane prior to cleavage of the thioglycoside anchor since it may cause side reactions in this last synthetic step. Activation of the C1–S-bond was performed by addition of NBS in the presence of ethanol as the glycosyl acceptor. The 6-O-carbamoyl-substituted ethyl glycosides **3** were formed in high purity and yield (Scheme 1).

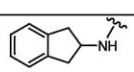
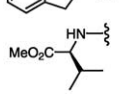
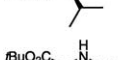
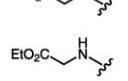
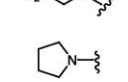
The aminolysis of the resin-bound imidazolides also proceeds with amino acid esters such as L-valine *tert*-butyl ester. Surprisingly, if polymer **2** was treated with mixtures of ethyl diisopropylamine (DIPEA) and amino acid ester hydrochlorides, no satisfactory conversion could be detected. Therefore, a more reactive phosgene equivalent had to be employed: Reaction of trichloromethyl chloroformate (diphosgene) with primary alcohols normally yields the corresponding chloroformates in a clean reaction. When applied to resin **1** however, even the presence of an excess of DIPEA could not prevent cleavage of the highly acid sensitive 1-ethoxyethyl protecting group and simultaneous conversion of both the 6- and the 4-hydroxy group to the chloroformate (Scheme 2). The resulting polymer **4** was reacted with pyrrolidine and several mixtures of amine hydrochlorides and DIPEA. With the bis-chloroformate, all reactions proceeded smoothly.[10] After cleavage of



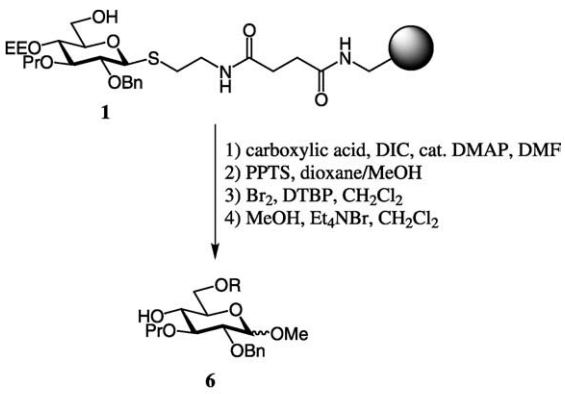
compound	R	crude yield	HPLC-purity (ELSD)
3-a		92%	85% (α)
3-b		74%	83% (α), 7% (β)
3-c		76%	86% (α), 4% (β)
3-d		65%	55% (α), 3% (β)
3-e		76%	82% (α), 8% (β)

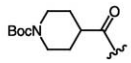
Scheme 1. Formation of carbamates via an imidazolide.



compound	R	crude yield	HPLC-purity (ELSD)
5-a		46%	86% (α), 4% (β)
5-b		49%	82% (α), 3% (β)
5-c		48%	77% (α), 4% (β)
5-d		54%	77% (α), 4% (β)
5-e		43%	70% (α), 4% (β)

Scheme 2. Formation of carbamates via chloroformates.



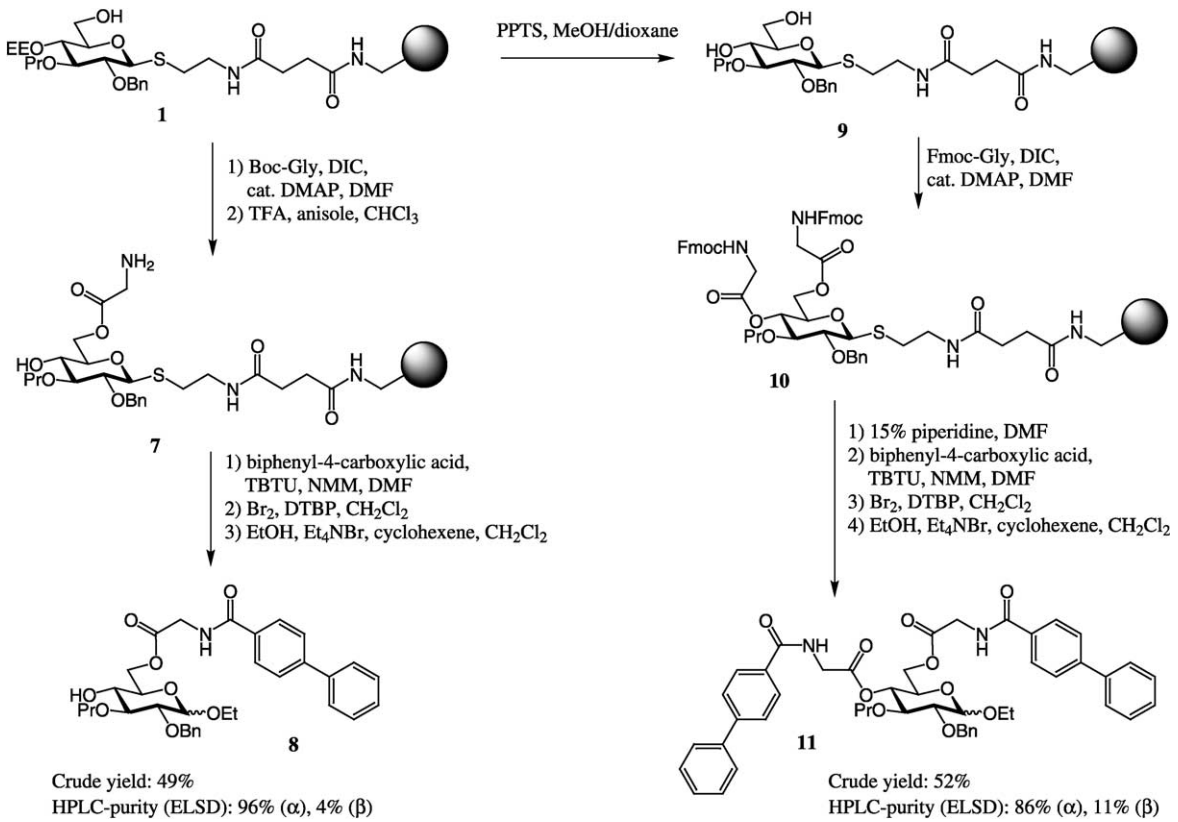
compound	R	crude yield	HPLC-purity (205-208 nm)
6-a	Boc-Gly-	44%	41% (α), 7% (β)
6-b	Boc-L-Ala-	57%	58% (α)
6-c	Boc-L-Pro-	62%	56% (α), 16% (β)
6-d	Boc-L-Phe-	66%	68% (α), 4% (β)
6-e		60%	60% (α), 10% (β)

Scheme 3. Esterification of Boc-protected amino acids.

the anchor and transglycosylation to ethanol, the bis-carbamates **5** were obtained.

Several esterification methods can be used for linking the C-terminus of an amino acid to one of the hydroxy functions of the carbohydrate scaffold. The Steglich esterfication with *N*,*N*′-dicyclohexylcarbodiimide and 4-dimethylamino-pyridine (DMAP) as a catalyst has been reported to give good yields for solid phase bound alcohols.[11,12] A modified Steglich protocol, in which *N*,*N*′-diisopropylcarbodiimide (DIC) is applied to avoid formation of an insoluble dialkyl urea in the reaction vessel and does not require cooling to 0 °C was employed for attaching various N-Boc-protected amino acids to template **1** (Scheme 3).

Neither the HPLC chromatograms of the products nor the ^{1}H NMR spectrum of compound **6-d** did show an undesired racemization. On the other hand, marked racemization could be detected when Boc-L-phenylalanine was linked to the 4-position of a 2,6-di-O-benzyl-3-O-propyl substituted glucose scaffold under identical conditions. The slower reaction with a secondary hydroxy group probably accounts for this difference. Using the coupling reagent 1-(mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT) which has been reported to be superior to other esterification reagents did not improve the results in this case.[13] In order to assess the possibility of performing reactions at the N-termini of amino acids C-terminally linked to the scaffold, resin **1** was esterified with Boc-glycine, the Boc group was cleaved with trifluoroacetic acid in chloroform. Anisole was added to the cleavage mixture to trap *tert*-butyl cations. The resin was washed with DIPEA/dioxane to liberate the free amine and 4-biphenylcarboxylic acid was coupled to the N-terminus using O-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU)[14] as the coupling reagent, 1-hydroxybenzotriazole (HOBt)[15] as the additive and N-methylmorpholine (NMM) as the



Scheme 4. Acylations of amino groups.

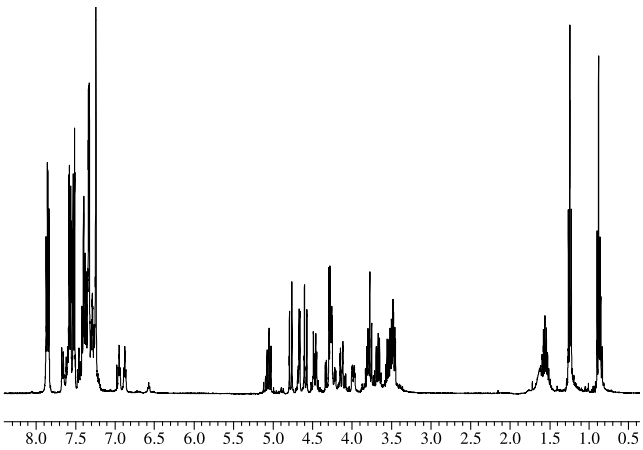


Figure 1. ^{1}H NMR spectrum of compound **11** (crude).

base. The resulting amide was detached from the resin under formation of the ethylglycoside **8** (Scheme 4). In order to test the parallel modification of both hydroxy groups and the stability of glycine esters of the glucose scaffold under the conditions for removal of the Fmoc-group, the ethoxyethyl group on template **1** was removed and the resulting 1,3-diol **9** was esterified with Fmoc-glycine, furnishing diester **10**. After removal of the Fmoc-groups by treatment with a solution of piperidine in DMF (15%), both N-termini were simultaneously condensed with biphenyl-4-carboxylic acid. After cleavage of the thioglycoside anchor, ethyl glycoside **11** was obtained in high purity. The ^{1}H NMR spectrum of crude compound **11** is shown in Figure 1.

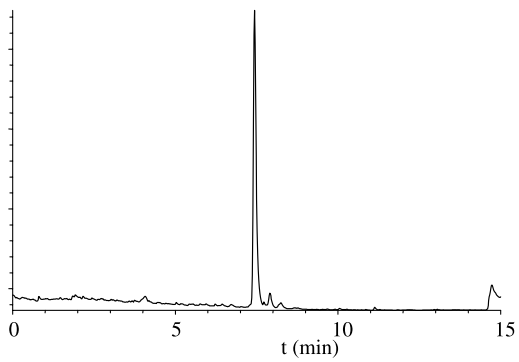
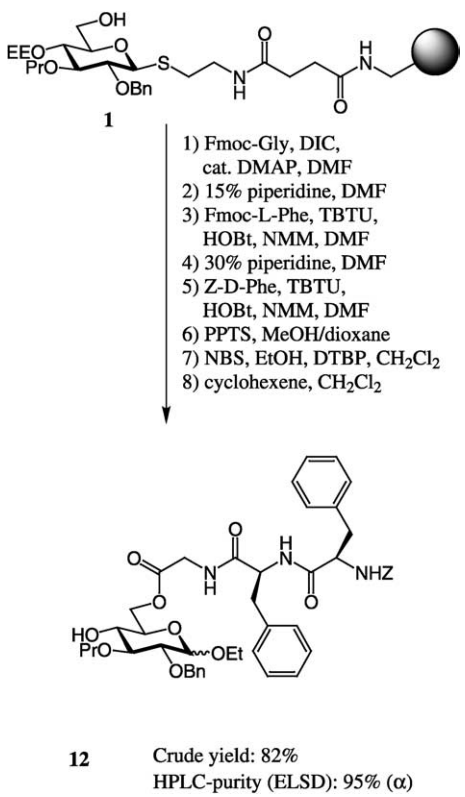
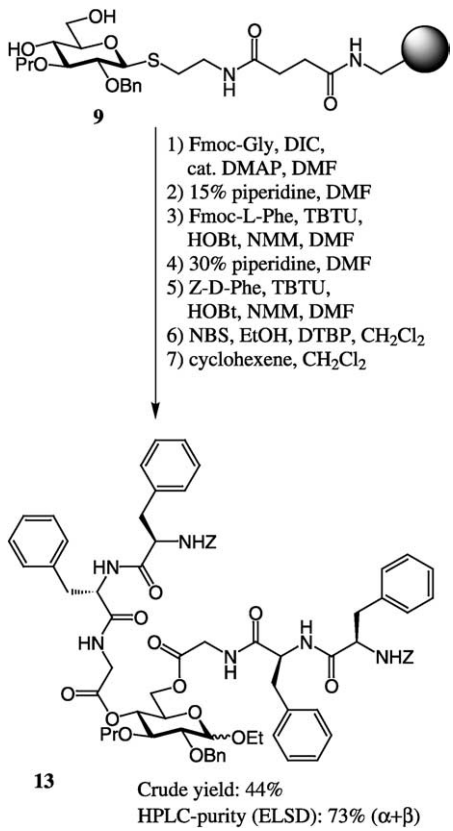


Figure 2. HPLC chromatogram of compound **12** (crude).

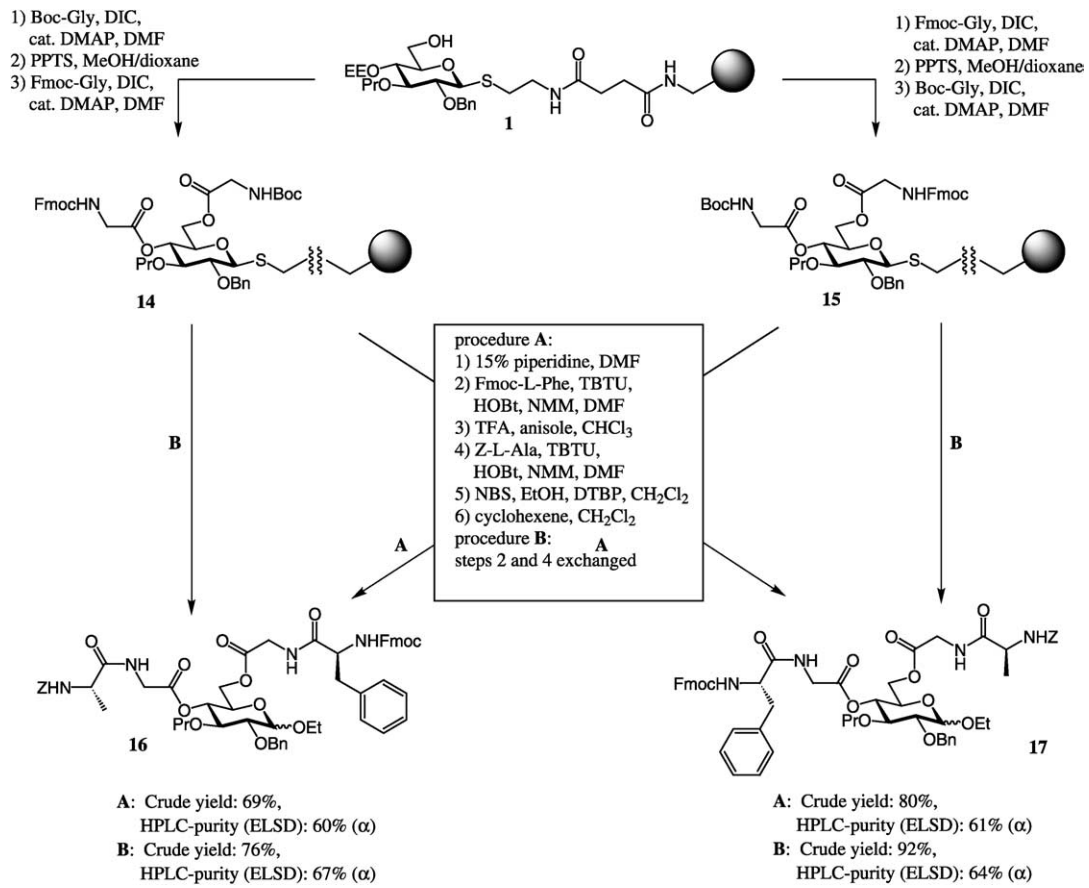
Based on these results, the synthesis of esters of the antiviral tripeptide Z-D-Phe-L-Phe-Gly-OH[16] as model compounds was attempted (Scheme 5): Fmoc-glycine was attached to the 6-OH-group of template **1** and the Fmoc-group was removed. After coupling of Fmoc-L-phenylalanine to the N-terminus using TBTU as the coupling reagent and HOBt, the Fmoc-group was cleaved off and Z-D-phenylalanine was linked to the N-terminus under identical conditions. For the removal of the second Fmoc-group, a higher concentration of piperidine in DMF (30%) in combination with a shorter reaction time was employed in order to minimize the risk of diketopiperazine formation.[17] Cleavage of the thiogyloside anchor in the presence of ethanol furnished ethyl glycoside-tripeptide ester **12** in high purity and yield. The HPLC-chromatogram of the crude material is shown in Figure 2.



Scheme 5. Preparation of tripeptide ester **12**.



Scheme 6. Preparation of bis-tripeptide ester **13**.

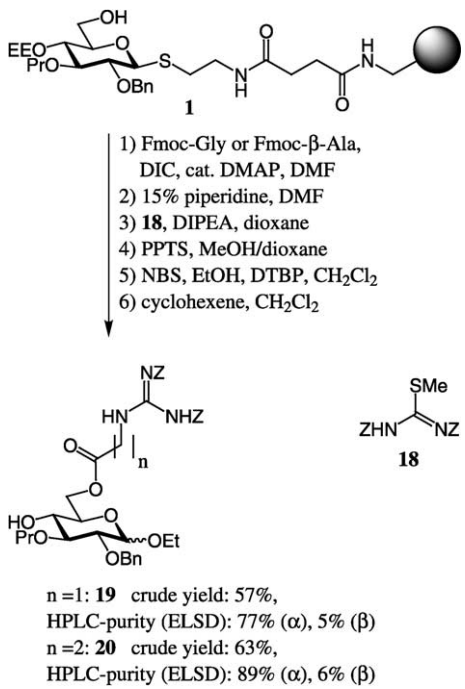


Scheme 7. Preparation of bis-dipeptide esters **16** and **17**.

In a similar manner, bis-tripeptide ester **13** was prepared from diol scaffold **9** (Scheme 6).

Since the Fmoc- and the Boc-group can be selectively cleaved in the presence of each other, not only the sequential attachment of two different side chains but also the sequential elongation of two peptide chains attached to the monosaccharide scaffold can be achieved. To demonstrate this potential, resin-bound glycosides **14** and **15** were prepared by attaching Fmoc-glycine to the 6-position and Boc-glycine to the 4-position and vice versa. The N-termini of both isomeric building blocks were selectively derivatized by removal of the Fmoc group, coupling Fmoc-L-phenylalanine, cleaving the Boc-group and coupling Z-L-alanine. Cleavage of the thioglycoside anchor in the presence of ethanol furnished the isomeric ethyl glycosides **16** and **17**. It is also possible to invert the order of the peptide couplings (variant B, see Scheme 7). In this case, the same pair of products was obtained in slightly higher yield and purity.

The analysis of the crude products **16** and **17** by HPLC-MS revealed that substantial amounts of the corresponding 1-OH glycosides were formed in the cleavage step. This side reaction is probably due to the ability of the polymer-bound bis-dipeptide structure to coordinate water molecules which were introduced with the coupling additive. Since 1-hydroxybenzotriazole is explosive in its dehydrated form, it is marketed with a water content of about 12%. The water which cannot be washed away from the resin before the cleavage of the anchor then competes with the glycosyl acceptor for the glycosyl bromide. The use of chemical drying (e.g. by addition of trimethyl orthoformate), a higher concentration of the glycosyl acceptor or a different

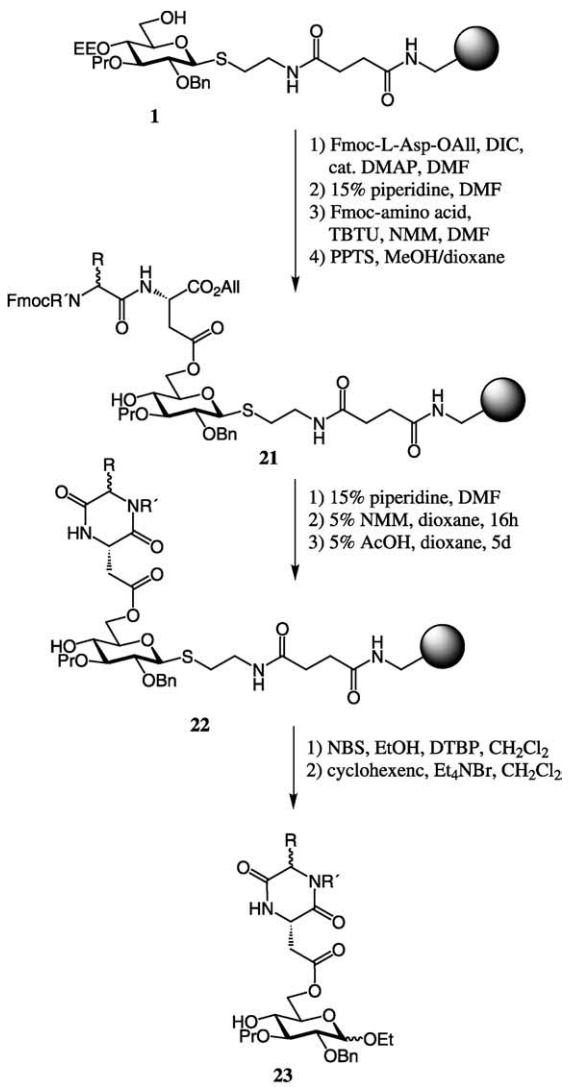


Scheme 8. Preparation of guanidines **19** and **20**.

coupling additive should suppress the formation of this undesired side product.

Apart from the formation of amides, free N-termini of amino acids linked to the glucose scaffold can also be converted into guanidines upon reaction with S-alkyl isothioureas. As an example, the bis-(benzyloxycarbonyl)-protected guanidines **19** and **20** were obtained when N,N′-bis-(benzyloxycarbonyl)-S-methylisothiourea **18** was employed as the guanylating reagent (Scheme 8).[18]

It is also possible to perform cylization reactions within the side chains attached to the monosaccharide core. By this strategy, the formation of products containing two polyfunctionalized heterocycles is possible. As an example, the 6-OH-group of scaffold **1** was esterified with the 4-carboxy group of Fmoc-L-aspartic acid 1-allyl ester. After removal of the Fmoc-group and elongation of the N-terminus to a dipeptide, diketopiperazines **23** were formed by intramolecular aminolysis in high purity and yield (see Scheme 9). Since basic[19] as well as acidic[20] conditions have been reported to accelerate the formation of diketopiperazines from dipetide esters, both were applied consecutively to the resin bound glycosides in order to achieve a complete conversion prior to cleavage from solid support.



compound	amino acid	crude yield	HPLC-purity (ELSD)
23-a	Fmoc-Gly	74%	90% (α)
23-b	Fmoc-L-Pro	80%	90% (α)
23-c	Fmoc-L-Phe	88%	94% (α)
23-d	Fmoc-D-Phe	74%	93% (α)

Scheme 9. Preparation of diketopiperazines **23**.

3. Conclusions

In summary, we demonstrate that amino acids can be attached to the hydroxy functions of the polyfunctional glucose template both via the N- or the C-terminus, giving rise to products of high structural diversity. Elongation at the N-terminus can be performed following the protocols of standard solid phase peptide synthesis using the Boc- or the Fmoc-strategy without affecting the linkage to the scaffold or the scaffold itself. By incorporation of two amino acids carrying orthogonally stable N-protecting groups, selective manipulations in the sidechains are possible. This strategy should also allow the construction of dendrimers with different arms. Finally, products with cyclic side chains can be obtained if trifunctional building blocks are attached to the monosaccharide core. These results suggest carbohydrate scaffolds as being favourable templates for the assembly of different peptide arms in the course of the combinatorial synthesis of artificial receptors.

4. Experimental

Analytical TLC was performed on aluminium backed TLC-plates coated with silica 60 F_{254} (E. Merck). Aminomethylated polystyrene was purchased from Rapp Polymere, Tübingen, Germany. Solid phase extraction cartridges and 13 mm polyethylene frits for standard 5 mL-polyethylene syringes were purchased from International Sorbent Technology (ict, Bad Homburg, Germany). NMR spectra were recorded on a Bruker AMX-400 spectrometer; chemical shifts were referenced to the $CHCl_3$-signal at 7.24 ppm and are expressed in ppm downfield from tetramethylsilane. ESI-MS spectra were measured on a Navigator instrument (ThermoQuest) between 200 and 1300 *m/z* (unless stated otherwise) at a cone voltage of 70 V using a flow rate of 0.75 mL/min acetonitrile/water 70:30 v/v and a nitrogen flow of 300 L/h. A Basic-Marathon autosampler was employed for sample injection (20 μL at 0.1 g/L in acetonitrile). ESI-HRMS analyses were performed on a Micromass Q-TOF Ultima 3 spectrometer. HPLC analyses were performed on a Knauer system with a Luna C18-2 column (Phenomenex, 75×4.6 mm, 3 μm particle size) using the following gradient: (*t*, %MeCN): 0 min, 50%; 0.75 min, 50%; 10 min, 99%; 12.5 min, 99%; 13.5 min, 50%; 15 min, 50%. For the analysis of compounds **23**, a different gradient was used: (*t*, %MeCN): 0 min, 25%; 0.75 min, 25%; 10 min, 99%; 12.5 min, 99%; 13.5 min, 25%; 15 min, 25%. The HPLC system was equipped with an autosampler,

an online-degasser, a column oven (25 °C), an ELS (evaporative light scattering) detector, model PL-ELS 1000 (Polymer Laboratories), and/or a UV detector (model 2140, LKB) operated at a wavelength of 205–208 nm. The ELS detector was operated at a nebulizer temperature of 85 °C and an evaporator temperature of 95 °C with a nitrogen flow of 1 L/min; for analytical HPLC, the flow rate was 1 mL/min, the sample concentration amounted 1 g/L and the injection volume was 20 μL. HPLC-MS analyses were performed on the Navigator instrument using the HPLC-system as described above in combination with a flow splitter (ratio 10:1) and a sample concentration of 0.1 g/L. For the notation of the ions of side products detected in mass spectra the numbering of the glucose skeleton is used. All substituents denoted are attached to the oxygen atoms at the mentioned positions with the exception of substituents at the anomeric center. All signals showed isotope patterns consistent with the composition of the indicated ion species and the corresponding charge state.

The preparation of the polymer **1** (2-OBn, 6-OH, capped amino functions) was described in an earlier publication.[5]

4.1. Preparation of carbamates 3

Five portions of the resin **1** (100 mg each, loading 0.53 mmol/g, 53 μmol) were weighed in 5 mL syringes equipped with polyethylene frits. To each of the syringes was added a solution of potassium *tert*-butanolate (10 mg, 89 μmol) in dioxane (0.2 mL). Immediately afterwards, a solution of 1,1′-carbonyldiimidazole (CDI, 200 mg, 1.23 mmol) in dioxane (1.5 mL) was added. The syringes were shaken for 3 h at room temperature and the resins were washed with dioxane (3 mL). Solutions of piperidine, pyrrolidine, morpholine, diethylamine and aminomethyl cyclopropane (0.2 mL each) in dioxane (1.5 mL each) were added to the resin portions and the syringes were shaken for 16 h at room temperature. The resins were washed with dioxane, DMF, dioxane and toluene (3 mL each). Then, the ethoxyethyl protecting group was removed by addition of a solution of pyridinium *p*-toluenesulfonate (PPTS, 22 mg, 88 μmol) in a mixture of methanol (0.2 mL) and dioxane (1.8 mL). After shaking for 10 min, the solution was discarded and the same amount of fresh PPTS-solution (identical composition) was filled in the syringes, followed by shaking for 16 h. The resins were washed with dioxane, DMF, dioxane, diethyl ether, dioxane, and twice with diethyl ether (2–3 mL each). Directly prior to cleavage of the thioglycoside anchor, the resin was washed with dry dichloromethane (1 mL). Detachment from the polymer was achieved by addition of a solution of N-bromosuccinimide (23.6 mg, 133 μmol), 2,6-di-*tert*-butylpyridine (36 μL, 160 μmol) and ethanol (200 μL, 3.4 mmol) in dry dichloromethane (2.5 mL) to all 12 syringes. After 20 min shaking, the solutions were removed from the syringes. Each of the solutions was filled in a vessel containing a solution of tetraethylammonium bromide (34 mg, 160 μmol) and cyclohexene (27 μL, 265 μmol) in dry dichloromethane (2 mL). Each resin sample was washed once with the resulting mixture in order to extract remaining glycosyl bromide and the vessels were closed. After 3 h, the caps were removed from the vessels and the volatile components were allowed to evaporate in a fume hood for 16 h. Five solid phase extraction cartridges (3 mL, with 20 μm polyethylene frit) were filled with silica (2 mL each) and the adsorbent was wetted with hexane (2–3 mL). The remaining compound mixtures were dissolved in dichloromethane (400 μL each) and applied on top of the silica layers. All nonpolar components were washed out with hexane (10 mL) and the desired products were eluted with a mixture of hexane and ethyl acetate (1:2, 7.5 mL). After removal of the solvents in vacuo, the obtained products **3** were weighed and analyzed by TLC, RP-HPLC and ESI-MS.

4.1.1. Ethyl 2-O-benzyl-6-O-(1-piperidinocarbonyl)-3-O-propyl-α/β-D-glucopyranoside 3-a. 20.9 mg (92%), colorless oil; R_f (α)=0.45 (hexane/EtOAc 1:1); $C_{24}H_{37}NO_7$ (451.6). HPLC (ELSD): 5.82 min (85.4%, α); 6.67 min (13.0%, β+impurity). ESI-MS (*m*/*z*)=925.6 (7%, $[2M+Na]^+$); 697.8 (6%, $[3M+Ca]^{2+}$); 564.4 (8%); 515.4 (11%, $[M+MeCN+Na]^+$); 492.3 (13%, $[2M+MeCN+Ca]^{2+}$); 474.3 (100%, $[M+Na]^+$), calcd: 474.3; 471.5 (25%, $[2M+Ca]^{2+}$); 463.4 (5%); 453.3 (8%, $[2,6\text{-}Bn_2+Na]^+$). ESI-HRMS: calcd for $C_{24}H_{37}NO_7+Na$: 474.2468, found: 474.2440.

A ^1H NMR spectrum of the crude product was recorded.

^1H NMR (400 MHz, $CDCl_3$): δ 7.37–7.25 (m, 5H, Ph), 4.75 (d, 1H, *J*=12.3 Hz, CH_2Ph), 4.69 (d, 1H, *J*=3.7 Hz, H-1), 4.59 (d, 1H, *J*=12.3 Hz, CH_2Ph), 4.54 (dd, 1H, $J_{6a,6b}$=12.5 Hz, $J_{5,6a}$=4.0 Hz, H-6a), 4.11 (dd, 1H, $J_{6a,6b}$=12.5 Hz, $J_{5,6b}$=1.9 Hz, H-6b), 3.87–3.25 (m, 12H, 2×OCH_2, 2×NCH_2, H-2–H-5), 1.75–1.42 (m, 8H, 3×CH_2 (piperidine), CH_2 (Pr)), 1.21 (t, 3H, *J*=7.1 Hz, CH_3 (Et)), 0.91 (t, 3H, *J*=7.4 Hz, CH_3 (Pr)). The spectrum showed the presence of succinimide (from the cleavage reaction) as a contaminant.

4.1.2. Ethyl 2-O-benzyl-6-O-(1-pyrrolidinocarbonyl)-3-O-propyl-α/β-D-glucopyranoside 3-b. 16.4 mg (74%), colorless oil; R_f (α)=0.32 (hexane/EtOAc 1:1); $C_{23}H_{35}NO_7$ (437.5). HPLC (ELSD): 4.57 min (83.0%, α); 5.30 min (6.5%, β); 6.67 min (7.8%). ESI-MS (*m*/*z*)=897.5 (7%, $[2M+Na]^+$); 676.2 (8%, $[3M+Ca]^{2+}$); 557.4 (6%); 501.3 (11%, $[M+MeCN+Na]^+$); 478.0 (26%, $[2M+MeCN+Ca]^{2+}$); 469.9 (5%); 460.3 (100%, $[M+Na]^+$), calcd: 460.2; 457.6 (27%, $[2M+Ca]^{2+}$); 453.3 (8%, $[2,6\text{-}Bn_2+Na]^+$); 449.6.

4.1.3. Ethyl 2-O-benzyl-6-O-(4-morpholinocarbonyl)-3-O-propyl-α/β-D-glucopyranoside 3-c. 17.4 mg (76%), colorless oil; R_f (α)=0.26 (hexane/EtOAc 1:1); $C_{23}H_{35}NO_8$ (453.5). HPLC-MS (ELSD): R_t, *m*/*z*=3.40 min (85.8%; 476.3, $[M(\alpha)+Na]^+$; 517.4, $[M(\alpha)+MeCN+Na]^+$); 4.02 min (3.9%; 476.3, $[M(\beta)+Na]^+$; 517.4, $[M(\beta)+MeCN+Na]^+$); 6.67 min (7.7%; 453.3, $[2,6\text{-}Bn_2(\alpha)+Na]^+$; 494.3, $[2,6\text{-}Bn_2(\alpha)+MeCN+Na]^+$). ESI-MS (*m*/*z*)=517.3 (10%, $[M+MeCN+Na]^+$); 494.1 (28%, $[2M+MeCN+Ca]^{2+}$); 486.0 (6%); 476.2 (100%, $[M+Na]^+$), calcd: 476.2; 473.3 (11%, $[2M+Ca]^{2+}$); 460.3 (12%); 453.3 (26%, $[2,6\text{-}Bn_2+Na]^+$). ESI-HRMS: calcd for $C_{23}H_{35}NO_8+Na$: 476.2260, found: 476.2236.

4.1.4. Ethyl 2-O-benzyl-6-O-(diethylcarbamoyl)-3-O-propyl-α/β-D-glucopyranoside 3-d. 14.4 mg (65%), colorless oil; R_f (α)=0.56 (hexane/EtOAc 1:1); $C_{23}H_{37}NO_7$ (439.5). HPLC (ELSD): 1.85 min (6.2%); 4.42 min (54.8%, α); 4.98 min (3.0%, β); 6.65 min (20.3%); 7.23 min (6.9%); 8.68 min (4.6%). ESI-MS (*m*/*z*)=565.4 (11%); 534.4 (12%); 525.4 (12%); 511.3 (10%); 507.3 (7%); 493.3 (72%); 476.2 (9%); 462.3 (100%, $[M+Na]^+$), calcd: 462.3; 453.3 (86%, $[2,6\text{-}Bn_2+Na]^+$); 434.3 (26%); 421.3 (50%). ESI-HRMS: calcd for $C_{23}H_{37}NO_7$+Na: 462.2468, found: 462.2467.

4.1.5. Ethyl 2-O-benzyl-6-O-(cyclopropylmethylcarbamoyl)-3-O-propyl-α/β-D-glucopyranoside 3-e. 16.7 mg (76%), colorless oil; R_f (α)=0.47 (hexane/EtOAc 1:1); $C_{23}H_{35}NO_7$ (437.5). HPLC (ELSD): 4.57 min (82.0%, α); 5.25 min (7.9%, β); 6.65 min (7.2%). ESI-MS (*m*/*z*)=676.8 (5%, $[3M+Ca]^{2+}$); 550.4 (9%); 501.3 (9%, $[M+MeCN+Na]^+$); 478.0 (20%, $[2M+MeCN+Ca]^{2+}$); 460.3 (100%, $[M+Na]^+$), calcd: 460.2; 457.6 (17%, $[2M+Ca]^{2+}$); 453.3 (14%, $[2,6\text{-}Bn_2+Na]^+$). ESI-HRMS: calcd for $C_{23}H_{35}NO_7$+Na: 460.2311, found: 460.2290.

4.2. Preparation of bis-carbamates 5

Five portions of the resin **1** (100 mg each, loading 0.53 mmol/g, 53 μmol) were weighed in 5 mL syringes equipped with polyethylene frits. To each of the syringes was added a solution of trichloromethyl chloroformate (60 μL, 0.5 mmol) and DIPEA (174 μL, 1 mmol) in 1.3 mL dioxane and the syringes were shaken for 3 h at room temperature. The polymers were washed with dioxane (3 mL) and solutions of DIPEA (174 μL, 1 mmol) and 2-aminoindane hydrochloride, L-valine methyl ester hydrochloride, glycine *tert*-butyl ester hydrochloride, glycin ethyl ester hydrochloride (0.75 mmol each) or pyrrolidine (0.75 mmol) in dry DMF (1.5 mL) were added. After shaking for 16 h at room temperature, the resins were washed with dioxane, DMF, dioxane and toluene (3 mL each). The ethoxyethyl protecting group was removed and the products were cleaved from the resin and isolated as described for the preparation of compounds **3**.

4.2.1. Ethyl 2-O-benzyl-4,6-bis-O-(2-indanylcarbamoyl)-3-O-propyl-α/β-D-glucopyranoside 5-a. 15.4 mg (46%), colorless crystals; R_f (α)=0.21 (hexane/EtOAc 3:1); $C_{38}H_{46}N_2O_8$ (658.8). HPLC (ELSD): 9.20 min (86.3%, α); 9.53 min (3.8%, β); 9.67 min (4.4%). ESI-MS (*m*/*z*)=1007.7 (7%); 681.5 (100%, $[M+Na]^+$), calcd: 681.3; 678.8 (24%, $[2M+Ca]^{2+}$); 670.8 (9%); 644.1 (7%); 612.3 (71%, $[2,6\text{-}Bn_2+Na]^+$); 522.3 (26%, $[4\text{-}OH+Na]^+$); 460.3 (6%); 453.3 (8%, $[2,6\text{-}Bn_2\text{-}4\text{-}OH+Na]^+$). ESI-HRMS: calcd for $C_{38}H_{46}N_2O_8$+Na: 681.3152, found: 681.3149.

4.2.2. Ethyl 2-O-benzyl-4,6-bis-O-((1*S*)-1-methoxycarbonyl-2-methyl-propylcarbamoyl)-3-O-propyl-α/β-D-glucopyranoside 5-b. 16.0 mg (49%), colorless oil; R_f (α)=0.18 (hexane/EtOAc 3:1); $C_{32}H_{50}N_2O_{12}$ (654.8). HPLC-MS (ELSD): R_t, *m*/*z*=5.38 min (4.4%; 649.4, $[1\text{-}OH(\alpha+\beta)+Na]^+$); 7.55 min (82.1%; 677.5, $[M(\alpha)+Na]^+$); 7.90 min (3.3%; 677.5, $[M(\beta)+Na]^+$); 8.92 min (6.3%; 610.3, $[2,6\text{-}Bn_2(\alpha)+Na]^+$). ESI-MS (*m*/*z*)=730.6 (6%); 693.4 (6%); 677.5 (100%, $[M+Na]^+$), calcd: 677.3; 674.5 (14%, $[2M+Ca]^{2+}$); 667.3 (5%); 660.7 (6%); 649.4 (12%, $[1\text{-}OH+Na]^+$); 610.3 (31%, $[2,6\text{-}Bn_2+Na]^+$); 520.4 (11%, $[4\text{-}OH+Na]^+$). ESI-HRMS: calcd for $C_{32}H_{50}N_2O_{12}$+Na: 677.3261, found: 677.3259.

4.2.3. Ethyl 2-O-benzyl-4,6-bis-O-(*tert*-butoxycarbonylmethylcarbamoyl)-3-O-propyl-α/β-D-glucopyranoside 5-c. 15.8 mg (48%), colorless waxy solid; R_f (α)=0.18 (hexane/EtOAc 3:1); $C_{32}H_{50}N_2O_{12}$ (654.8). HPLC (ELSD): 5.32 min (6.9%); 6.02 min (3.6%); 8.05 min (77.2%, α); 8.48 min (4.1%, β); 9.10 min (5.3%). ESI-MS (*m*/*z*)=775.4 (9%); 677.5 (100%, $[M+Na]^+$), calcd: 677.3; 649.4 (8%); 621.3 (30%, $M-C_4H_8+Na]^+$); 565.3 (53%); 562.5 (11%); 554.4 (25%); 521.3 (8%); 480.2 (5%); 464.2 (17%). ESI-HRMS: calcd for $C_{32}H_{50}N_2O_{12}$+Na: 677.3261, found: 677.3268.

4.2.4. Ethyl 2-O-benzyl-4,6-bis-O-(ethoxycarbonylmethylcarbamoyl)-3-O-propyl-α/β-D-glucopyranoside 5-d. 16.2 mg (54%), colorless oil; R_f (α)=0.45 (hexane/EtOAc 1:1); $C_{28}H_{42}N_2O_{12}$ (598.6). HPLC-MS (ELSD): R_t, *m*/*z*=3.83 min (7.7%; 492.3, $[4\text{-}OH(\alpha)+Na]^+$); 5.43 min (76.9%; 621.3, $[M(\alpha)+Na]^+$); 5.92 min (4.1%; 621.3, $[M(\beta)+Na]^+$); 7.75 min (6.4%; 582.3, $[2,6\text{-}Bn_2(\alpha)+Na]^+$). ESI-MS (*m*/*z*)=621.3 (100%, $[M+Na]^+$), calcd: 621.3; 618.6 (24%, $[2M+Ca]^{2+}$); 593.3 (9%); 582.3 (23%, $[2,6\text{-}Bn_2+Na]^+$); 492.3 (14%, $[4\text{-}OH+Na]^+$); 474.2 (9%). ESI-HRMS: calcd for $C_{28}H_{42}N_2O_{12}$+Na: 621.2635, found: 621.2607.

4.2.5. Ethyl 2-O-benzyl-4,6-bis-O-(1-pyrrolidinocarbonyl)-3-O-propyl-α/β-D-glucopyranoside 5-e. 11.5 mg (43%), colorless waxy solid; R_f (α)=0.35 (hexane/EtOAc 1:1); $C_{28}H_{42}N_2O_8$ (534.7). HPLC (ELSD): 4.55 min (14.4%); 6.67 min (3.4%); 7.27 min (70.4%, α); 7.80 min (4.3%, β); 9.23 min (5.6%). ESI-MS (*m*/*z*)=621.3 (6%); 591.3 (6%); 557.4 (100%, $[M+Na]^+$), calcd: 557.3; 554.8 (14%, $[2M+Ca]^{2+}$); 550.4 (35%, $[2,6\text{-}Bn_2+Na]^+$); 460.3 (33%, $[4\text{-}OH+Na]^+$); 420.4 (7%). ESI-HRMS: calcd for $C_{28}H_{42}N_2O_8$+Na: 557.2839, found: 557.2839.

4.3. Preparation of compounds 6

Five portions of the resin **1** (75 mg each, loading 0.45 mmol/g, 34 μmol) were weighed in 5 mL syringes equipped with polyethylene frits. To each of the syringes was added a solution of Boc-glycine, Boc-L-alanine, Boc-L-proline, Boc-L-phenylalanine or Boc-piperidine-4-carboxylic acid (340 μmol each), N,N′-diisopropylcarbodiimide (64 μL, 410 μmol) and DMAP (3 mg, 25 μmol) in dry DMF (1.5 mL). The syringes were shaken for 16 h at room temperature and the resins were washed with DMF, dioxane, DMF, dioxane and diethyl ether (3 mL each). After removal of the ethoxyethyl protecting group (for the protocol, see preparation of compounds **3**), the thioglycoside achor was cleaved: The resin samples were washed with dry dichloromethane (1 mL) and a solution of bromine (17.5 μL, 340 μmol) and 2,6-di-*tert*-butylpyridine (112 μL, 500 μmol) in dry dichloromethane (1.3 mL) was added. The syringes were shaken for 1 h. A solution of tetraethylammonium bromide (40 mg, 190 μmol), cyclohexene (177 μL, 1.75 mmol) and methanol (300 μL, 7.4 mmol) in dry dichloromethane (1 mL) was added. Shaking was continued for 2 h and the solutions containing the desired

O-glycosides were removed from the syringes through the frits by pushing the plungers in. Each resin portion was washed with dry dichloromethane (2 mL), the solution was combined with the reaction mixture from the corresponding syringe, and the volatile components were allowed to evaporate in a fume hood for 16 h. The solid phase extraction of compounds **6** was performed as described for compounds **3**. The products were eluted from the silica-cartridges with hexane/ethyl acetate (2:3, 7.5 mL).

4.3.1. Methyl 2-O-benzyl-6-O-(N-*tert*-butoxycarbonyl-glycyl)-3-O-propyl-α/β-D-glucopyranoside 6-a. 6.7 mg (44%), weakly orange oil; R_f (α)=0.26 (hexane/EtOAc 2:1); $C_{24}H_{37}NO_9$ (483.6). HPLC (UV): 2.72 min (4.9%); 4.56 min (41.0%, α); 5.22 min (6.9%, β); 5.72 min (20.5%, 2,6-Bn_2); 6.33 min (8.2%, β-2,6-Bn_2); 7.30 min (5.6%); 7.92 min (3.4%). ESI-MS (*m*/*z*)=989.5 (6%, $[2M+Na]^+$); 668.5 (6%); 552.3 (13%, $[M+K]^+$); 506.3 (100%, $[M+Na]^+$), calcd: 506.2; 480.2 (10%, $[2,6\text{-}Bn_2+MeCN+Na]^+$); 463.2 (8%); 450.2 (87%, $[M-C_4H_8+Na]^+$); 439.2 (16%, $[2,6\text{-}Bn_2+Na]^+$); 406.4 (25%, $[M-Boc+Na]^+$); 385.2 (11%). ESI-HRMS: calcd for $C_{24}H_{37}NO_9+Na$: 506.2366, found: 506.2372.

4.3.2. Methyl 2-O-benzyl-6-O-(N-*tert*-butoxycarbonyl-L-alanyl)-3-O-propyl-α/β-D-glucopyranoside 6-b. 8.9 mg (57%), weakly orange oil; R_f (α)=0.38 (hexane/EtOAc 2:1); $C_{25}H_{39}NO_9$ (497.6). HPLC (UV): 5.22 min (57.9%, α); 5.77 min (20.4%, β+2,6-Bn_2); 6.38 min (3.5%, β-2,6-Bn_2); 7.53 min (4.3%); 7.73 min (4.3%); 7.93 min (3.8%); 13.53 min (4.3%). ESI-MS (*m*/*z*)=1017.5 (8%, $[2M+Na]^+$); 682.5 (7%); 592.3 (9%, $[M+C_4H_8O+Na]^+$); 536.3 (13%, $[M+K]^+$); 520.4 (100%, $[M+Na]^+$), calcd: 520.4; 464.2 (90%, $[M-C_4H_8+Na]^+$); 420.4 (35%, $[M-Boc+Na]^+$). ESI-HRMS: calcd for $C_{25}H_{39}NO_9+Na$: 520.2523, found: 520.2499.

4.3.3. Methyl 2-O-benzyl-6-O-(N-*tert*-butoxycarbonyl-L-prolyl)-3-O-propyl-α/β-D-glucopyranoside 6-c. 10.2 mg (62%), weakly orange oil; R_f (α)=0.26 (hexane/EtOAc 2:1); $C_{27}H_{41}NO_9$ (523.6). HPLC (UV): 5.75 min (8.3%, 2,6-Bn_2); 5.93 min (56.1%, α); 6.65 min (15.9%, β); 9.50 min (3.3%). ESI-MS (*m*/*z*)=1069.6 (8%, $[2M+Na]^+$); 708.5 (7%); 562.4 (10%, $[M+K]^+$); 546.4 (100%, $[M+Na]^+$), calcd: 546.4; 490.2 (90%, $[M-C_4H_8+Na]^+$); 446.3 (44%, $[M-Boc+Na]^+$). ESI-HRMS: calcd for $C_{27}H_{41}NO_9+Na$: 546.2679, found: 546.2659.

4.3.4. Methyl 2-O-benzyl-6-O-(N-*tert*-butoxycarbonyl-L-phenylalanyl)-3-O-propyl-α/β-D-glucopyranoside 6-d. 11.8 mg (66%), weakly yellowish oil; R_f (α)=0.50 (hexane/EtOAc 2:1); $C_{31}H_{43}NO_9$ (573.7). HPLC-MS: R_t, *m*/*z*=5.75 min (6.7%; 439.4, $[2,6\text{-}Bn_2(\alpha)+Na]^+$; 480.4, $[2,6\text{-}Bn_2(\alpha)+MeCN+Na]^+$); 7.33 min (68.3%; 596.4, $[M(\alpha)+Na]^+$; 540.5, $[M(\alpha)-C_4H_8+Na]^+$; 496.4, $[M(\alpha)-Boc+Na]^+$); 7.93 min (3.5%; 596.4, $[M(\beta)+Na]^+$; 540.5, $[M(\beta)-C_4H_8+Na]^+$; 496.4, $[M(\beta)-Boc+Na]^+$). ESI-MS (*m*/*z*)=1169.5 (8%, $[2M+Na]^+$); 612.3 (6%, $[M+K]^+$); 596.3 (100%, $[M+Na]^+$), calcd: 596.5; 540.4 (80%, $[M-C_4H_8+Na]^+$); 496.3 (31%, $[M-Boc+Na]^+$); 493.8 (11%, $[2\times(M-Boc)+Ca]^{2+}$). ESI-HRMS: calcd for $C_{31}H_{43}NO_9+Na$: 596.2836, found: 596.2848.

A ^1H NMR spectrum of the crude product was recorded.

^1H NMR (400 MHz, $CDCl_3$): δ 7.11–7.36 (m, 2×Ph, $CHCl_3$), 4.94 (d, br, 1H, $J_{NH\text{-}CH}$=7.4 Hz, NH), 4.74 (d, 1H, J_{gem}=12.1 Hz, OCH_2 (Bn)), 4.56–4.68 (m, OCH_2 (Bn), contaminant), contained in this multiplet: 4.60 (d, 1H, J_{gem}=12.1 Hz, OCH_2 (Bn)) and 4.53 (d, 1H, $J_{1,2}$=3.5 Hz, H-1), 4.29–4.32 (m, 2H, H_2-6), 3.89 (dt, 1H, J_d=9 Hz, J_t=7 Hz, OCH_2 (Pr)), 3.73 (dψt, 1H, J_d=9.8 Hz, J_t≈3.7 Hz, H-5), 3.62 (dt, 1H, J_d=9 Hz, J_t=7 Hz, OCH_2 (Pr)), 3.51–3.59 (m, 2H, H-4, Phe-Hα), 3.36 (dd, 1H, $J_{2,3}$=9.6 Hz, $J_{1,2}$=3.5 Hz, H-2), 3.32 (s, 3H, OMe), 3.26 (ψt, 1H, J≈9.6 Hz, H-3), 3.07 (m, br, 2H, Phe-Hβ), 1.55–1.66 (m, 2H, 2-CH_2 (Pr)), 1.38 (s, 9H, *t*Bu), 0.92 (t, 3H, *J*=7 Hz, CH_3 (Pr)).

4.3.5. Methyl 2-O-benzyl-6-O-(N-*tert*-butoxycarbonyl-piperidin-4-carbonyl)-3-O-propyl-α/β-D-glucopyranoside 6-e. 10.1 mg (60%), weakly yellowish oil; R_f (α)=0.28 (hexane/EtOAc 2:1); $C_{28}H_{43}NO_9$ (537.6). HPLC (UV): 5.77 min (10.5%, 2,6-Bn_2); 6.37 min (59.5%, α); 7.03 min (9.6%, β); 8.02 min (5.4%). ESI-MS (*m*/*z*)=1097.7 (8%, $[2M+Na]^+$); 632.4 (13%, $[M+C_4H_8O+Na]^+$); 576.4 (8%, $[M+K]^+$); 560.4 (100%, $[M+Na]^+$), calcd: 560.4; 557.8 (9%, $[2M+Ca]^{2+}$); 545.5 (7%, $[M-C_4H_8+MeCN+Na]^+$); 504.3 (39%, $[M-C_4H_8+Na]^+$); 501.5 (6%, $[M-Boc+MeCN+Na]^+$); 460.3 (38%, $[M-Boc+Na]^+$); 439.2 (8%, $[2,6\text{-}Bn_2+Na]^+$); 406.4 (6%).

4.3.6. Ethyl 2-O-benzyl-6-O-(N-(4-phenylbenzoyl)-glycyl)-3-O-propyl-α/β-D-glucopyranoside 8. In a 5 mL syringe equipped with a polyethylene frit, a portion of the polymer **1** (50 mg, loading 0.46 mmol/g, 23 μmol) was shaken with a solution of Boc-glycine (43.8 mg, 250 μmol), N,N′-diisopropylcarbodiimide (39.1 μL, 250 μmol) and DMAP (2.1 mg, 17 μmol) in dry DMF (1.5 mL) for 16 h at room temperature. The resin was washed with dioxane, DMF, dioxane and diethyl ether (3 mL each) and the Boc and the ethoxyethyl group were removed by shaking the resin with a solution of trifluoroacetic acid (250 μL) and anisole (100 μL) in chloroform (750 μL) for 1 h at room temperature. The resin was washed with dioxane, DMF, dioxane, diethyl ether and toluene. In order to neutralize the protonated amino functions, the polymer was shaken with a 10%-solution of DIPEA in dioxane for 10 min at room temperature. After addition of a solution of biphenyl-4-carboxylic acid 49.6 mg (250 μmol, 10.9 equiv.), O-(benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium tetrafluoroborate (TBTU, 80.3 mg, 250 μmol), 1-hydroxybenzotriazole (38.4 mg, contains 12% water, 250 μmol) and N-methylmorpholine (55 μL, 500 μmol) in dry DMF (1 mL); the resin was shaken for 1 h at room temperature. The polymer was washed with dioxane, DMF, methanol, DMF, dioxane, diethyl ether, dioxane, diethyl ether, toluene (3 mL each) and finally with dry dichloromethane (1 mL). For cleavage of the thioglycoside anchor, the polymer was shaken with a solution of bromine (5.1 μL, 100 μmol) and 2,6-di-*tert*-butylpyridine (50 μL, 223 μmol) in dry dichloromethane (1 mL) for 10 min at room temperature. The cleavage reaction was stopped by pouring the reaction mixture in a solution of tetraethylammonium bromide (37.5 mg, 178 μmol), cyclohexene (25 μL, 247 μmol) and ethanol (250 μL, 4.3 mmol) in dry dichloromethane (1 mL)

and the polymer was washed with the resulting mixture. After 3 h, the cap was removed from the vessel and the volatile components were allowed to evaporate in a fume hood for 16 h. The solid phase extraction was performed as described for compounds **3**. The product was eluted with a mixture of hexane and ethyl acetate (1:2, 7.5 mL).

5.8 mg (49%), colorless oil; R_f (α)=0.50 (PE/EE=1/1); $C_{33}H_{39}NO_8$ (577.7). HPLC-MS (ELSD): R_t, m/z=6.25 min (95.9%; 600.2, $[M(\alpha)+Na]^+$); 6.71 min (4.1%; 600.2, $[M(\beta)+Na]^+$). ESI-MS (m/z)=621.2 (5%); 600.1 (100%, $[M+Na]^+$), calcd: 600.3; 597.5 (11%, $[2M+Ca]^{2+}$); 572.2 (8%, $[1\text{-}OH+Na]^+$); 453.2 (10%, $[2,6\text{-}Bn_2+Na]^+$). ESI-HRMS: calcd for $C_{33}H_{39}NO_8$+Na: 600.2573, found: 600.2568.

4.3.7. Ethyl 2-O-benzyl-4,6-bis-O-(N-(4-phenylbenzoyl)-glycyl)-3-O-propyl-α/β-D-glucopyranoside 11. From a portion of the polymer **1** (50 mg, loading 0.53 mmol/g), the ethoxyethyl group was removed according to the protocol described for the preparation of compounds **3**. The resulting resin (50 mg, loading ca. 0.53 mmol/g, 26.5 μmol) was shaken with a solution of Fmoc-glycine (111.5 mg, 375 μmol), N,N′-diisopropylcarbodiimide (66.5 μL, 425 μmol) and DMAP (3.0 mg, 25 μmol) in dry DMF (1.5 mL) for 16 h at room temperature. The polymer was washed with DMF, dioxane, diethyl ether, dioxane, diethyl ether and toluene (3 mL each). The Fmoc groups were cleaved by shaking the resin with a 15% solution of piperidine in dry DMF for 10 min at room temperature. The resin was washed with dioxane, DMF, dioxane, diethyl ether and dioxane. Acylation of the N-termini was achieved by shaking the polymer with a solution of biphenyl-4-carboxylic acid (74.4 mg, 375 μmol), TBTU (120.5 mg, 375 μmol) and N-methylmorpholine (82.5 μL, 750 μmol) in dry DMF (1.5 mL) for 1 h at room temperature. Washing of the polymer and cleavage of the thioglycoside anchor as well as purification via solid phase extraction were performed as described for compound **8**.

10.1 mg (52%), colorless crystals; R_f (α)=0.56 (Tol/EtOH 4:1); $C_{48}H_{50}N_2O_{10}$ (814.9). HPLC (ELSD): 9.47 min (86.0%, α); 9.85 min (10.6%, β). ESI-MS (m/z)=1241.7 (6%); 837.3 (100%, $[M+Na]^+$), calcd: 837.3; 834.8 (30%, $[2M+Ca]^{2+}$); 827.3 (6%); 690.3 (45%, $[2,6\text{-}Bn_2+Na]^+$); 600.1 (10%); 435.2 (5%). ESI-HRMS: calcd for $C_{48}H_{50}N_2O_{10}$+Na: 837.3363, found: 837.3351.

A ^{1}H NMR spectrum of the crude product was recorded.

^{1}H NMR+COSY (400 MHz, $CDCl_3$): δ 7.85, 7.84 (2t, 2×2H, J=6.7 Hz, biphenyl-*o*-H_2), 7.68–7.49 and 7.47–7.25 (m, 19H, aryl), 6.94 (t, br, 1H, J=5.3 Hz, NH), 6.87 (t, br, 1H, J=5.3 Hz, NH), 5.05 (ψt, 1H, $J\approx$10 Hz, H-4), 4.77 (d, 1H, d, 1H, J=12.1 Hz, CH_2Ph), 4.66 (d, 1H, J=3.7 Hz, H-1), 4.59 (d, 1H, J=12.1 Hz, CH_2Ph), 4.47 (dd, br, 1H, $J_{6a,6b}$=12.3 Hz, $J_{5,6a}$=4.2 Hz, H-6a), 4.34–4.20 (m, 4H, Gly-CH_2), 4.13 (dd, br, 1H, $J_{6a,6b}$=12.3 Hz, $J_{5,6b}$=2.5 Hz, H-6b), 3.98 (ddd, 1H, $J_{4,5}$=10.2 Hz, $J_{5,6a}$=4.2 Hz, $J_{5,6b}$=2.5 Hz, H-5), 3.83–3.74 (m, 2H, H-3, OCH_2 (Pr)), 3.67 (dq, 1H, J_{gem}=10.0 Hz, J_{vic}=7.1 Hz, OCH_2 (Et)), 3.58–3.44 (m, 3H, OCH_2 (Pr), OCH_2 (Et), H-2), 1.66–1.44 (m, 2H, CH_2 (Pr)), 1.24 (t, 3H, J=7.1 Hz, CH_3 (Et)), 0.88 (t, 3H, J=7.4 Hz, CH_3 (Pr)).

4.3.8. Ethyl 2-O-benzyl-6-O-(N-benzyloxycarbonyl-D-phenylalanyl-L-phenylalanyl-glycyl)-3-O-propyl-α/β-D-glucopyranoside 12. In a 5 mL syringe equipped with a polyethylene frit, a portion of the polymer **1** (50 mg, loading 0.46 mmol/g, 23 μmol) was shaken with a solution of Fmoc-glycine (74 mg, 245 μmol), N,N′-diisopropylcarbodiimide (39 μL, 250 μmol) and DMAP (2 mg, 17 μmol) in dry DMF (1.5 mL) for 16 h at room temperature. The resin was washed with DMF, dioxane, diethyl ether, dioxane, diethyl ether and toluene and the Fmoc group was removed as described for the preparation of compound **11**. The N-terminus was acylated by shaking the resin with a solution of Fmoc-L-phenylalanine (48 mg, 125 μmol), TBTU (40 mg, 125 μmol), 1-hydroxybenzotriazole (22 mg, contains 12% water, 125 μmol) and N-methylmorpholine (27.5 μL, 250 μmol) in dry DMF (1 mL) for 50 min at room temperature. The polymer was washed with DMF, dioxane, diethyl ether, dioxane, diethyl ether and toluene. The Fmoc group was cleaved by shaking the resin with a 30% solution of piperidine in dry DMF (1 mL) for 3 min at room temperature. The resin was washed quickly with DMF (2×), dioxane and DMF. The final acylation was achieved by shaking the polymer with a solution of Z-D-phenylalanine (52 mg, 175 μmol), TBTU (56 mg, 175 μmol), 1-hydroxybenzotriazole (30 mg, contains 12% water, 175 μmol) and N-methylmorpholine (39 μL, 350 μmol) in dry DMF (1 mL) for 2 h at room temperature. The polymer was washed with DMF, dioxane, diethyl ether, dioxane, diethyl ether and toluene and the ethoxyethyl group was removed according to the protocol described for the preparation of compounds **3**. Cleavage of the thioglycoside anchor and the subsequent solid phase extraction were also performed according to the protocol for the preparation of compounds **3**. However, no tetraethylammonium bromide was added to the reaction mixture. The compound was eluted from the silica gel cartridge with hexane/ethyl acetate (1:3, 7.5 mL).

14.0 mg (82%), colorless crystals; R_f (α)=0.14 (hexane/EtOAc 1:1); $C_{46}H_{55}N_3O_{11}$ (826.0). HPLC-MS (ELSD): R_t, m/z=7.43 min (95.2%; 848.3, $[M(\alpha)+Na]^+$). ESI-MS (m/z)=995.3 (13%); 864.3 (6%, $[M+K]^+$); 848.3 (100%, $[M+Na]^+$), calcd: 848.4; 820.3 (6%, $[1\text{-}OH+Na]^+$); 758.2 (15%, $[M-C_7H_6+Na]^+$). ESI-HRMS: calcd for $C_{46}H_{55}N_3O_{11}$+Na: 848.3734, found: 848.3738.

A ^{1}H NMR spectrum of the crude product was recorded.

^{1}H NMR+COSY (400 MHz, $CDCl_3$): δ 7.34–7.14 and 7.08–7.00 (m, aryl-H, $CHCl_3$), 6.91 (t, br, 1H, $J\approx$6 Hz, Gly-NH), 6.78 (d, br, 1H, J=8.4 Hz, Phe^2-NH), 5.77 (d, br, 1H, J=7.4 Hz, NHZ), 5.02 (d, 1H, d, 1H, J=12.3 Hz, CH_2Ph (Z)), 4.96 (d, 1H, d, 1H, J=12.3 Hz, CH_2Ph (Z)), 4.71–4.62 (m, 3H, H-1, Phe^2-Hα, CH_2Ph (Bn)), 4.52 (d, 1H, J=12.1 Hz, CH_2Ph (Bn)), 4.38 (dd, br, 1H, $J_{6a,6b}\approx$12 Hz, $J_{5,6a}\approx$4 Hz, H-6a), 4.32–4.24 (m, 2H, Phe^1-Hα, H-6b), 4.00 (dd, 1H, J_{gem}=18.0 Hz, J_{vic}=6.2 Hz, Gly-CH_2), 3.94–3.59 (m, 6H, OCH_2 (Pr), OCH_2 (Et, 1H), H-3, H-5, Gly-CH_2 (1H)), 3.50–3.35 (m, 3H, H-2, H-4, OCH_2 (Et, 1H)), contained in this multiplet: 3.37 (dd, 1H,

$J_{2,3}$=9.5 Hz, $J_{1,2}$=3.6 Hz, H-2), 2.97–2.82 (m, 4H, Phe-Hβ), 1.64–1.54 (m, 2H, CH_2 (Pr)), 1.20 (t, 3H, J=7.2 Hz, CH_3 (Et)), 0.89 (t, 3H, J=7.0 Hz, CH_3 (Pr)). The spectrum showed the presence of succinimide (from the cleavage reaction) as a contaminant.

4.3.9. Ethyl 2-O-benzyl-4,6-bis-O-(N-(benzyloxycarbonyl)- D-phenylalanyl-L-phenylalanyl-glycyl)-3-O-propyl-α/β-D-glucopyranoside 13. The synthesis was performed according to the protocol for the preparation of compound **11**. The acylations of the N-termini of the glycine residues (attachment of Fmoc-L-Phe and Z-D-Phe) were performed as described for the preparation of compound **12** using the 1.5-fold amount of all reagents. Cleavage from the resin and solid phase extraction were also performed analogously.

13.8 mg (44%), colorless crystals; R_f (α)=0.25 (hexane/EtOAc 1:1); $C_{74}H_{82}N_6O_{16}$ (1311.5). HPLC-MS (ELSD): R_t, m/z=7.70 min (3.1%; 910.3, [2,6-Bn_2-1-OH(α)+Na]$^+$); 9.10 min (11.8%; 1305.6, [1-OH(α+β)+Na]$^+$); 9.85 min (6.0%; 938.4, [2,6-Bn_2(α)+Na]$^+$); 10.17 min (73.2%; 1333.7, [M(α+β)+Na]$^+$; 1369.5, [1-Br(α)+Na]$^+$); 10.52 min (3.2%; 1480.6). ESI-MS (200–1600u, m/z)=1480.5 (27%); 1452.5 (17%); 1369.5 (10%); 1333.7 (94%, [M+Na]$^+$), calcd: 1333.6; 1305.6 (42%, [1-OH+Na]$^+$); 1243.4 (10%, [M−C_7H_6+Na]$^+$); 1085.4 (10%); 938.4 (100%, [2,6-Bn_2+Na]$^+$); 910.3 (23%, [2,6-Bn_2-1-OH+Na]$^+$); 848.4 (46%, [2,6-Bn_2-C_7H_6+Na]$^+$); 749.0 (16%); 734.5 (12%); 675.5 (30%); 662.2 (18%); 542.2 (17%); 526.2 (45%); 435.2 (13%); 390.1 (11%); 210.2 (14%). ESI-HRMS: calcd for $C_{74}H_{82}N_6O_{16}$+Na: 1333.5685, found: 1333.5699.

4.3.10. Ethyl 2-O-benzyl-4-O-(N-benzyloxycarbonyl-L-alanyl-glycyl)-6-O-(N-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl-glycyl)-3-O-propyl-α/β-D-glucopyranoside 16. *Variant A*. In a 5 mL syringe equipped with a polyethylene frit, a portion of the polymer **1** (50 mg, loading 0.46 mmol/g, 23 μmol) was shaken with a solution of Fmoc-glycine (74 mg, 245 μmol), N,N′-diisopropylcarbodiimide (39 μL, 250 μmol) and DMAP (2 mg, 17 μmol) in dry DMF (1.5 mL) for 16 h at room temperature. The resin was washed with DMF, dioxane, diethyl ether, dioxane, diethyl ether and toluene (3 mL each) and the ethoxyethyl group was removed as described for compounds **3**. A second Steglich-esterification was carried out under identical conditions using Boc-glycine (44 mg, 250 μmol) instead of Fmoc-glycine. The polymer was washed with DMF, dioxane, DMF, dioxane, diethyl ether and toluene. The Fmoc group was removed as described for the preparation of compound **11** and the resin was shaken with a solution of Fmoc-L-phenylalanine (48 mg, 125 μmol), TBTU (40 mg, 125 μmol), 1-hydroxybenzotriazole (22 mg, contains 12% water, 125 μmol) and N-methylmorpholine (27.5 μL, 250 μmol) in dry DMF (1 mL) for 15 min at room temperature. The resin was washed with DMF, dioxane, diethyl ether, dioxane, diethyl ether and toluene (3 mL each). The Boc group was removed by shaking the resin with a solution of trifluoroacetic acid (250 μL) and anisole (100 μL) in chloroform (750 μL) for 1 h at room temperature. The resin was washed with dioxane, DMF, dioxane, diethyl ether and toluene. In order to neutralize the protonated amino functions, the polymer was shaken with a 10%-solution of DIPEA in dioxane for 10 min at room temperature. The free N-terminus was acylated by shaking the polymer with a solution of Z-L-alanine (39 mg, 175 μmol), TBTU (56 mg, 175 μmol), 1-hydroxybenzotriazole (30 mg, contains 12% water, 175 μmol) and N-methylmorpholine (58 μL, 525 μmol)in dry DMF (1 mL) for 2 h at room temperature. The polymer was washed with dioxane, DMF, dioxane, diethyl ether, dioxane, diethyl ether and toluene. Cleavage of the thioglycoside anchor and isolation of the product were performed as described for compound **12**.

14.8 mg (69%), colorless crystals; R_f (α)=0.19 (hexane/EtOAc 1:1); $C_{57}H_{64}N_4O_{14}$ (1029.2). HPLC (ELSD): 7.95 min (38.6%, 1-OH, α+β); 9.35 min (60.1%, α). ESI-MS (m/z)=1198.4 (7%); 1067.2 (6%, [M+K]$^+$); 1051.3 (100%, [M+Na]$^+$), calcd: 1051.4; 1034.2 (24%); 1023.2 (47%, [1-OH+Na]$^+$); 1006.2 (15%); 887.2 (36%, [6-Z-Ala-Gly+Na]$^+$); 859.2 (13%, [6-Z-Ala-Gly-1-OH+Na]$^+$); 715.2 (55%, [2,6-Bn_2+Na]$^+$); 687.3 (20%, [2,6-Bn_2-1-OH+Na]$^+$); 625.1 (10%); 435.2 (19%); 303.1 (41%). ESI-HRMS: calcd for $C_{57}H_{64}N_4O_{14}$+Na: 1051.4317, found: 1051.4301.

Variant B. The preparation was performed according to variant A. However, the order of the first two acylations was reversed. After the second acylation with Fmoc-glycine (4-position), the Fmoc group was removed as described for compound **11**. The N-terminus was acylated by shaking the resin with a solution of Z-L-alanine (28 mg, 125 μmol, TBTU (40 mg, 125 μmol), 1-hydroxybenzotriazole (22 mg, contains 12% water, 125 μmol) and N-methylmorpholine (27.5 μL, 250 μmol) in dry DMF (1 mL) for 50 min at room temperature. The polymer was washed with DMF, dioxane, diethyl ether, dioxane, diethyl ether and toluene. The Boc group was removed as described in variant A, and the N-terminus was acylated by shaking the resin with a solution of Fmoc-L-phenylalanine (68 mg, 175 μmol), TBTU (56 mg, 175 μmol), 1-hydroxybenzotriazole (30 mg, contains 12% water, 175 μmol) and N-methylmorpholine (58 μL, 525 μmol) in dry DMF (1 mL) for 2 h at room temperature. The polymer was washed with dioxane, DMF, dioxane, diethyl ether, dioxane, diethyl ether and toluene. Cleavage of the thioglycoside anchor and isolation of the product was performed as described for compound **12**.

16.2 mg (76%), colorless crystals; R_f (α)=0.20 (hexane/EtOAc 1:1); $C_{57}H_{64}N_4O_{14}$ (1029.2). HPLC-MS (ELSD): R_t, m/z=7.98 min (32.6%; 1023.3, [1-OH(α)+Na]$^+$); 9.38 min (67.4%; 1051.3, [M(α)+Na]$^+$). ESI-MS (m/z)=1198.4 (8%); 1051.3 (100%, [M+Na]$^+$), calcd: 1051.4; 1034.2 (6%); 1023.2 (44%, [1-OH+Na]$^+$); 887.2 (18%, [6-Z-Ala-Gly+Na]$^+$); 859.2 (8%, [6-Z-Ala-Gly-1-OH+Na]$^+$); 715.2 (24%, [2,6-Bn_2+Na]$^+$); 687.3 (10%, [2,6-Bn_2-1-OH+Na]$^+$); 607.2 (5%); 435.2 (15%); 303.1 (37%).

4.3.11. Ethyl 2-O-benzyl-6-O-(N-benzyloxycarbonyl-L-alanyl-glycyl)-4-O-(N-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl-glycyl)-3-O-propyl-α/β-D-glucopyranoside 17. *Variant A*. The preparation was performed

analogously to the synthesis of compound **16**, variant A. However, the order of the first two acylations was reversed (see preparation of compound **16**, variant B). After the second acylation with Fmoc-glycine (4-position), the Fmoc group was removed as described for compound **11**. Couplings to the N-terminus, including removal of the Boc-group, cleavage of the thioglycoside anchor and solid phase extraction were performed according to the protocol for the preparation of compound **16**, variant A.

17.0 mg (80%), colorless crystals; R_f (α)=0.22 (hexane/EtOAc 1:1); $C_{57}H_{64}N_4O_{14}$ (1029.2). HPLC (ELSD): 8.08 min (30.8%, 1-OH, α); 9.42 min (60.6%, α). ESI-MS (*m*/*z*)=1198.4 (12%); 1170.3 (6%); 1051.3 (100%, $[M+Na]^+$), calcd: 1051.4; 1034.2 (33%); 1023.2 (50%, $[1\text{-}OH+Na]^+$); 1006.2 (19%); 887.2 (8%); 879.2 (12%); 862.3 (7%); 467.1 (10%); 450.1 (7%); 287.1 (7%).

Variant B. The preparation was performed according to the synthesis of compound **16**, variant A. After the second acylation with Boc-glycine (4-position), the Fmoc group was removed as described for the preparation of compound **11**. Couplings to the N-terminus, including removal of the Boc-group, cleavage of the thioglycoside anchor and solid phase extraction were performed according to the protocol for the preparation of compound **16**, variant B.

19.5 mg (92%), colorless crystals; R_f (α)=0.22 (hexane/EtOAc 1:1); $C_{57}H_{64}N_4O_{14}$ (1029.2). HPLC-MS (ELSD): R_t, *m*/*z*=8.12 min (30.4%; 1023.3, $[1\text{-}OH(\alpha)+Na]^+$); 9.43 min (63.8%; 1051.3, $[M(\alpha)+Na]^+$). ESI-MS (*m*/*z*)=1198.4 (8%); 1067.3 (7%, $[M+K]^+$); 1051.3 (100%, $[M+Na]^+$), calcd: 1051.4; 1023.2 (48%, $[1\text{-}OH+Na]^+$); 961.4 (8%, $[M-C_7H_6+Na]^+$); 933.3 (5%, $[1\text{-}OH-C_7H_6+Na]^+$); 789.3 (5%, $[2,6\text{-}Bn_2+Na]^+$); 467.1 (10%). ESI-HRMS: calcd for $C_{57}H_{64}N_4O_{14}$+Na: 1051.4317, found: 1051.4277.

4.3.12. Ethyl 2-O-benzyl-6-O-((2,3-bis-benzyloxycarbonyl)-guanidino)-acetyl-3-O-propyl-α/β-D-glucopyranoside 19. In a 5 mL syringe equipped with a polyethylene frit, a portion of the polymer **1** (50 mg, loading 0.46 mmol/g, 23 μmol) was shaken with a solution of Fmoc-glycine (74 mg, 245 μmol), N,N′-diisopropylcarbodiimide (39 μL, 250 μmol) and DMAP (2 mg, 17 μmol) in dry DMF (1.5 mL) for 16 h at room temperature. The resin was washed with DMF, dioxane, diethyl ether, dioxane, diethyl ether and toluene and the Fmoc group was removed as described for the preparation of compound **11**. The polymer was shaken with a solution of N,N′-bis-(benzyloxycarbonyl)-*S*-methyl-isothiourea (45 mg, 125 μmol) and DIPEA (4.3 μL, 25 μmol) in dioxane (1.5 mL) for 16 h at room temperature. Another portion of DIPEA (21 μL, 125 μmol) in dioxane (200 μL) was added to the syringe and the resin was shaken for another 2 h at room temperature. After washing the polymer with DMF, dioxane, diethyl ether, dioxane, diethyl ether and toluene, the ethoxyethyl group was removed according to the procedure for the synthesis of compounds **3**. The product was detached from the polymer by treatment with a solution of N-bromosuccinimide (11 mg, 62 μmol), and 2,6-di-*tert*-butylpyridine (50 μL, 223 μmol) in a 5% solution of ethanol in dichloromethane (1 mL) for 15 min at room temperature. The reaction was quenched by addition of the mixture to a solution of cyclohexene (60 μL, 590 μmol) in dry dichloromethane (1 mL). The polymer was washed with the resulting mixture and the reaction vessel was closed. After 3 h at room temperature, the volatile components were allowed to evaporate in a fume hood. The solid phase extraction of the product was performed as described for the preparation of compounds **3**.

8.3 mg (57%), colorless oil; R_f (α)=0.45 (hexane/EtOAc 1:1); $C_{37}H_{45}N_3O_{11}$ (707.8). HPLC-MS (ELSD): R_t, *m*/*z*=6.72 min (11.7%; 453.2, $[2,6\text{-}Bn_2(\alpha)+Na]^+$; 494.2, $[2,6\text{-}Bn_2(\alpha)+MeCN+Na]^+$); 7.30 min (6.9%; $[2,6\text{-}Bn_2(\beta)+Na]^+$); 9.07 min (76.8%; 730.2, $[M(\alpha)+Na]^+$; 622.2, $[M(\alpha)-C_7H_8O+Na]^+$; 579.2, $[M(\alpha)-(Z\text{-}NH_2)+Na]^+$; 535.2, $[M(\alpha)-(Z\text{-}NH_2)\text{-}CO_2+Na]^+$; 514.2, $[M(\alpha)-2\times C_7H_8O+Na]^+$); 9.53 min (4.6%; 730.2, $[M(\beta)+Na]^+$; 622.2, $[M(\beta)-C_7H_8O+Na]^+$; 579.2, $[M(\beta)-(Z\text{-}NH_2)+Na]^+$; 535.2, $[M(\beta)-(Z\text{-}NH_2)-CO_2+Na]^+$; 514.2, $[M(\beta)-2\times C_7H_8O+Na]^+$). ESI-MS (*m*/*z*)=730.1 (87%, $[M+Na]^+$), calcd: 730.3; 622.1 (92%, $[M-C_7H_8O+Na]^+$); 609.2 (12%); 595.2 (18%); 579.2 (30%, $[M-(Z\text{-}NH_2)+Na]^+$); 535.3 (72%, $[M-(Z\text{-}NH_2)-CO_2+Na]^+$); 514.1 (75%, $[M-2\times C_7H_8O+Na]^+$); 510.2 (13%); 461.2 (12%); 453.2 (100%, $[2,6\text{-}Bn_2+Na]^+$); 420.2 (23%); 363.2 (11%); 298.1 (14%). ESI-HRMS: calcd for $C_{37}H_{45}N_3O_{11}$+Na: 730.2952, found: 730.2931.

4.3.13. Ethyl 2-O-benzyl-6-O-(2-(2,3-bis-benzyloxycarbonyl)-guanidino)-propionyl-3-O-propyl-α/β-D-glucopyranoside 20. The preparation was performed according to the synthesis of compound **19**. However, Fmoc-β-alanine (77.8 mg, 250 μmol) was used instead of Fmoc-glycine.

9.4 mg (63%), colorless oil; R_f (α)=0.42 (hexane/EtOAc 1:1); $C_{38}H_{47}N_3O_{11}$ (721.8). HPLC-MS (ELSD): R_t, *m*/*z*=7.28 min (3.5%; 453.2, $[2,6\text{-}Bn_2(\beta)+Na]^+$; 716.3, $[1\text{-}OH(\alpha)+Na]^+$; 608.2, $[1\text{-}OH(\alpha)-C_7H_8O+Na]^+$; 565.3, $[1\text{-}OH(\alpha)-(Z\text{-}NH_2)+Na]^+$; 521.3, $[1\text{-}OH(\alpha)-(Z\text{-}NH_2)\text{-}CO_2+Na]^+$); 9.18 min (88.7%; 744.2, $[M(\alpha)+Na]^+$; 636.2, $[M(\alpha)-C_7H_8O+Na]^+$; 593.2, $[M(\alpha)-(Z\text{-}NH_2)+Na]^+$; 549.4, $[M(\alpha)-(Z\text{-}NH_2)-CO_2+Na]^+$; 528.3, $[M(\alpha)-2\times C_7H_8O+Na]^+$); 9.65 min (6.0%; 744.2, $[M(\beta)+Na]^+$; 636.2, $[M(\beta)-C_7H_8O+Na]^+$; 593.2, $[M(\beta)-(Z\text{-}NH_2)+Na]^+$; 549.4, $[M(\beta)-(Z\text{-}NH_2)-CO_2+Na]^+$; 528.3, $[M(\beta)-2\times C_7H_8O+Na]^+$). ESI-MS (*m*/*z*)=744.1 (80%, $[M+Na]^+$), calcd: 744.3; 722.2 (17%, $[M+H]^+$); 716.2 (10%); 636.1 (100%, $[M-C_7H_8O+Na]^+$); 633.7 (12%, $[2\times(M-C_7H_8O)+Ca]^{2+}$); 609.2 (26%); 593.2 (78%, $[M-(Z\text{-}NH_2)+Na]^+$); 524.2 (19%); 474.1 (8%); 453.2 (30%, $[2,6\text{-}Bn_2+Na]^+$); 434.2 (18%). ESI-HRMS: calcd for $C_{38}H_{47}N_3O_{11}$+Na: 744.3108, found: 744.3131.

4.4. Preparation of compounds 23

Four portions of the resin **1** (50 mg each, loading 0.46 mmol/g, 23 μmol) were weighed in 5 mL syringes equipped with polyethylene frits. To each of the syringes was added a solution of Fmoc-L-aspartic acid 1-allyl ester (96.9 mg, 245 μmol), N,N′-diisopropylcarbodiimide (39.1 μL, 250 μmol) and DMAP (2.1 mg, 17 μmol) in dry DMF (1.5 mL) and the syringes were shaken for 16 h at

room temperature. The resins were washed with DMF, dioxane, diethyl ether, dioxane, diethyl ether and toluene (3 mL each) and the Fmoc group was removed as described for the preparation of compound **11**. The polymers were shaken with solutions of TBTU (40.3 mg, 126 μmol), 1-hydroxybenzotriazole (21.7 mg, contains 12% water, 125 μmol), N-methylmorpholine (27.5 μL, 250 μmol) and Fmoc-glycine, Fmoc-L-proline, Fmoc-L-phenylalanine or Fmoc-D-phenylalanine (125 μmol) in dry DMF (1 mL) for 1.5 h at room temperature. The resins were washed with dioxane, DMF, dioxane, diethyl ether, dioxane, diethyl ether and toluene (3 mL each) and the ethoxyethyl group was removed according to the protocol for the preparation of compounds **3**. Cleavage of the Fmoc-group was achieved by shaking the polymers with a 15% solution of piperidine in DMF (1 mL each) for 30 min at room temperature. The polymers were washed twice with dioxane (3 mL each) and shaken with a 5% solution of N-methylmorpholine in dioxane for 16 h at room temperature. After washing with dioxane, DMF and dioxane (3 mL each), the polymers were shaken with a 5% solution of acetic acid in dioxane (1 mL each) for 5 d at room temperature. The resins were washed with dioxane, ethanol, DMF, dioxane, diethyl ether, dioxane, diethyl ether, toluene and dry dichloromethane (3 mL each). Cleavage of the thioglycoside anchor and solid phase extraction of the products was performed as described for the preparation of compounds **3**. The products were eluted from the silica gel cartridges with toluene/ethanol (4:1, 7.5 mL each).

4.4.1. Ethyl 2-O-benzyl-6-O-(((3*S*)-piperazin-2,5-dion-3-yl)-acetyl)-3-O-propyl-(α/β)-D-glucopyranoside 23-a. 7.6 mg (74%), weakly yellowish solid; R_f (α)=0.28 (Tol/EtOH 4:1); $C_{24}H_{34}N_2O_9$ (494.5). HPLC (ELSD): 4.87 min (89.8%, α); 5.23 min (5.2%); 9.07 min (3.1%). ESI-MS (*m*/*z*)=672.2 (5%); 539.2 (5%); 533.2 (18%, $[M+K]^+$); 517.2 (100%, $[M+Na]^+$), calcd: 517.2; 514.5 (20%, $[2M+Ca]^{2+}$); 453.2 (10%, $[2,6\text{-}Bn_2+Na]^+$). ESI-HRMS: calcd for $C_{24}H_{34}N_2O_9$+Na: 517.2162, found: 517.2159.

4.4.2. Ethyl 2-O-benzyl-6-O-((3*S*,8*aS*)-(1,4-dioxo-octahydro-pyrrolo[1,2-a]pyrazin-3-yl)-acetyl)-3-O-propyl-(α/β)-D-glucopyranoside 23-b. 8.9 mg (80%), weakly yellowish solid; R_f (α)=0.48 (Tol/EtOH 4:1); $C_{27}H_{38}N_2O_9$ (534.6). HPLC (ELSD): 5.63 min (89.6%, α); 9.10 min (4.5%). ESI-MS (*m*/*z*)=712.2 (10%); 657.2 (28%); 573.2 (11%, $[M+K]^+$); 557.3 (100%, $[M+Na]^+$), calcd: 557.3; 554.6 (8%, $[2M+Ca]^{2+}$); 453.2 (14%, $[2,6\text{-}Bn_2+Na]^+$); 335.1 (11%). ESI-HRMS: calcd for $C_{27}H_{38}N_2O_9$+Na: 557.2475, found: 557.2480.

4.4.3. Ethyl 2-O-benzyl-6-O-(((3*S*,6*S*)-6-benzylpiperazin-2,5-dion-3-yl)-acetyl)-3-O-propyl-(α/β)-D-glucopyranoside 23-c. 10.7 mg (88%), colorless crystals; R_f (α)=0.47 (Tol/EtOH 4:1); $C_{31}H_{40}N_2O_9$ (584.7). HPLC (ELSD): 6.35 min (93.7%, α). ESI-MS (*m*/*z*)=762.2 (7%); 623.2 (14%, $[M+K]^+$); 607.1 (100%, $[M+Na]^+$), calcd: 607.3; 604.5 (13%, $[2M+Ca]^{2+}$); 579.2 (6%); 517.2 (9%); 453.2 (10%, $[2,6\text{-}Bn_2+Na]^+$); 252.2 (6%); 210.1 (8%). ESI-HRMS: calcd for $C_{31}H_{40}N_2O_9$+Na: 607.2632, found: 607.2614.

4.4.4. Ethyl 2-O-benzyl-6-O-(((3*S*,6*R*)-6-benzylpiperazin-2,5-dion-3-yl)-acetyl)-3-O-propyl-(α/β)-D-glucopyranoside 23-d. 9.0 mg (74%), colorless crystals; R_f (α)=0.54 (Tol/EtOH 4:1); $C_{31}H_{40}N_2O_9$ (584.7). HPLC-MS (ELSD): R_t, *m*/*z*=6.38 min (93.3%; 607.2, $[M(\alpha)+Na]^+$); 9.12 min (4.3%; 453.2, $[2,6\text{-}Bn(\alpha)+Na]^+$). ESI-MS (*m*/*z*)=623.2 (17%, $[M+K]^+$); 607.2 (68%, $[M+Na]^+$), calcd: 607.3; 557.3 (14%); 467.6 (42%); 311.7 (18%); 289.2 (16%); 261.5 (100%). ESI-HRMS: calcd for $C_{31}H_{40}N_2O_9$+Na: 607.2632, found: 607.2606.

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Solid phase synthesis of an extensively focused library of thiadiazole ethers

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Abstract—An approach to combine the advantages of random and of focused combinatorial libraries in pharmaceutical research is described with the example of a solid phase synthesis of 2,5-disubstituted thiadiazole ethers. Key steps of synthesis are the introduction of the heterocycle by selective, sequential nucleophilic double substitution of 2,5-bis(methylsulfonyl)-1,3,4-thiadiazole and the oxidation of the benzylsulfanyl-1,3,4-thiadiazole to the corresponding sulfone using MCPBA on solid phase.

1. Introduction

Solid phase chemistry has emerged as a powerful tool for synthesis of small organic molecules useful for drug discovery. In pharmaceutical research solid phase chemistry has two major applications: (1) focused libraries allow for screening of structure activity relationship (SAR) in ongoing medicinal chemistry projects in one or more dimensions in parallel and (2) random libraries can cover a broad chemical space to provide compounds for future screening. A drawback of the first approach is that a focused library is often used just for the current target. Since large parts of the library have potency for this target, applicability is limited if selective hits are to be found for other targets in general screening. Furthermore, in current medicinal chemistry projects SAR is often only interesting in one dimension, whereas in other dimensions, SAR is already well established. Therefore, solid phase chemistry with its potential of varying several dimensions in parallel is only seldom fully applicable in current projects with partially developed SAR. On the other hand, the second approach of random libraries has to make sure to produce drug-like compounds since chemical space is much broader than space of biologically active molecules.[1,2] Biased libraries are one approach to circumvent these problems. These resemble random libraries which are expected to have potency for certain target classes.[3] We use a strategy of extensively focused libraries to avoid the problems and to combine the advantages of random and of focused libraries. In this approach, only part of the library is designed to cover the current SAR of a specific medicinal chemistry project whereas another part of the library covers additional chemical space for future screening. Since we know already about the efficacy of the scaffold in a medicinal chemistry project we have thereby reassured that the compounds synthesised are drug-like and highly valuable for screening. The following example illustrates these differences between focused, random and extensively focused libraries in part of chemical space (rectangle) where potent compounds in a given project are within the grey zone. Focused libraries are expected to lie mainly closely around the zone of potent compounds (a). Random libraries cover larger parts of chemical space, but only a few—if at all—are expected to lie within the zone of potent compounds (b). Extensively focused libraries cover a larger part of chemical space and a subset of these can be used to generate SAR information for the given target. A hit in another cluster in a new project can provide fast SAR-information (c) (Fig. 1).

Recently we were dealing with thiadiazoles which are important heterocyclic scaffolds of compounds which

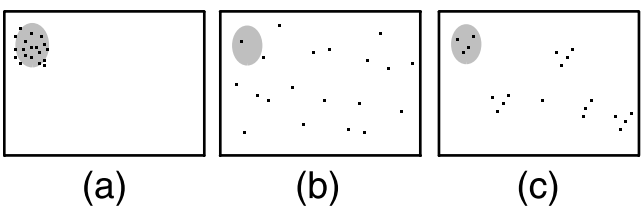

Figure 1. Illustration of the differences between focused (a), random (b) and extensively focused (c) libraries.

Keywords: Parallel synthesis; Solid phase synthesis; Sulfones; Thiadiazoles; Extensively focused libraries.
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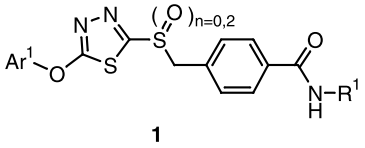
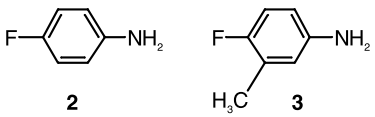
display a wide range of different biological activities. They play a role as cyclooxygenase inhibitors,[4] and carbonic anhydrase inhibitors[5] and are found in antibacterials like cefazolin.[6] In the course of our investigations for the prophylaxis of myocardial reinfarction we were studying antagonists of the Interleukin 8 receptor (IL-8) and we found benzylic thiadiazole sulfones **1** (Fig. 2, n=2) to be potent inhibitors of IL-8.



1

Figure 2. Scaffold of the library synthesised.

We were especially interested in the SAR and the potential of the 'left side' aryl substituents Ar^1 whereas we knew already about the high potential of the sulfone group (n=2) and of potent R^1-groups, the 4-fluorophenyl amides which are derived from anilines **2** and **3** (Fig. 3). Therefore, we used the synthesis to incorporate additional diversity in the right amide part and by synthesising thiadiazolyl thioethers (n=0) for general screening libraries.



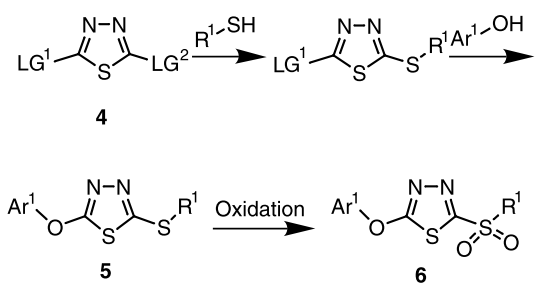
2 **3**

Figure 3. Anilines R^1-NH_2 essential in the right amide part of **1** for high potency versus IL-8.

2. Results and discussion

2.1. Synthesis of key intermediate (8)

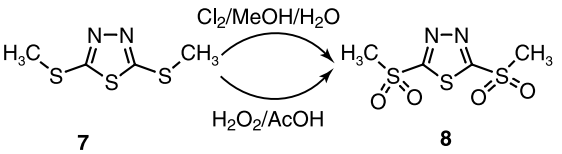
For incorporation of the heterocyclic scaffold we needed a thiadiazole structure **4** which was set up for double nucleophilic substitution. Double nucleophilic substitution can be performed conveniently on solid phase taking advantage of its inherent tendency to react only once with a substrate due to the pseudo dilution effect. This allows sequential introduction of the sulfur moiety and of the aryl ether. Subsequent oxidation of the thioether would yield the final products **6** (Scheme 1). Stopping the sequence before oxidation yields thioethers **5**.



4 **5** **6**

Scheme 1. Generation of thiadiazolyl sulfone ethers.

From former experiments we had recognised bis(methylsulfonyl)thiadiazole **8** as possible intermediate (–LG^1=–LG^2=–SO_2CH_3). However, literature synthesis performed oxidation of **7** with gaseous chlorine in methanol/water mixture and separated **8** from mono-oxidised intermediates and from substrate **7** by preparative chromatography (Scheme 2).[7]



7 **8**

Scheme 2. Possible ways for generation of intermediate **8**.

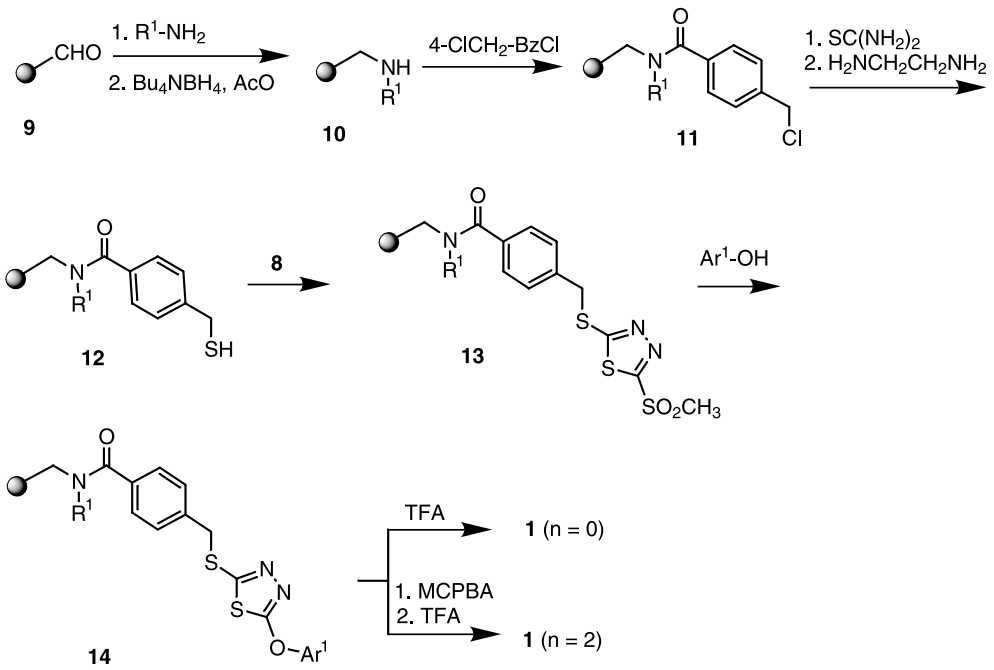
We found that **7** can be cleanly oxidised with H_2O_2 in acetic acid and isolated by precipitation with water. This method avoids handling of gaseous chlorine and evaporative concentration of possible peroxide species after oxidation and is highly convenient to produce multigram quantities of bis(methylsulfonyl)thiadiazole for combinatorial synthesis.

2.2. Library synthesis

The combinatorial synthesis of thiadiazolyl sulfones was carried out using IRORI Mini Kan technology.[8] We chose to use 4-(4-formyl-3-methoxyphenoxy)butyryl aminomethyl resin **9** (aldehyde resin, NovaBiochem) for our reaction sequence which allows straightforward synthesis of solid phase bound amines and anilines for further derivatisation and which can be easily cleaved with trifluoroacetic acid (Scheme 3).

In the first place, n-propylamine and 4-fluoroaniline **2** were reductively alkylated with aldehyde resin **9** (NovaBiochem) by imine formation and subsequent reduction of the imines with tetrabutylammonium borohydride. These solid phase bound substrates **10** were reacted with 4-chloromethylbenzoylchloride and diisopropylethylamine (DIEA) in dichloromethane to give the corresponding amides **11**. The benzylic halides were transferred into the thiols **12** by a two step procedure:[9] reaction with thiourea gave rise to the alkylated isothiourea which was cleaved with ethylene diamine in a second step. The thiol derivatives **12** proved stable over night in the refrigerator but slowly decomposed even on solid phase at room temperature on contact with air by formation of disulfides as analysed by LC-MS.

Reaction of **8** with solid phase bound thiol using DMAP as a base gave cleanly intermediate **13**. The second sulfone group was replaced smoothly with a set of phenols (Fig. 4) using Cs_2CO_3 as a base to gain thiadiazolyl ethers **14**. Omitting the next step for the first half of the library at this stage provided thiodiazolyl ethers (**1**, n=0). Oxidation of the other half with 3-chloroperbenzoic acid provided thiadiazolyl sulfones (**1**, n=2). LC-MS-analysis showed that formation of the sulfoxides (**1**, n=1) was performed within a few minutes whereas the further oxidation yielding the sulfones took 4–5 h. The proceeding of this reaction was monitored carefully on the basis of selected compounds since too long reaction times resulted in slow degradation of the final products. All compounds **1** were cleaved off the solid phase with trifluoroacetic acid, evaporated to dryness and analysed by LC-MS. The purity of the final products was for most of the compounds above 70% and exceeded an average of 85% as detected by LC-MS (see Table 1).



Scheme 3. Library synthesis.

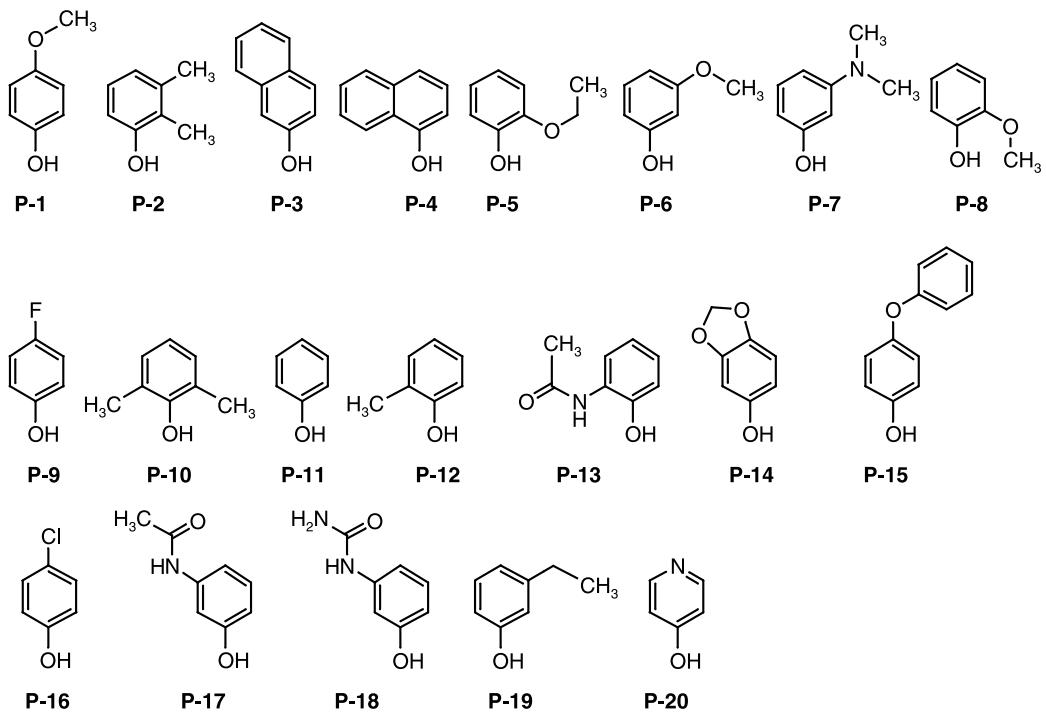


Figure 4. Phenols used in the first library for SAR-development.

Identity was confirmed with exception of products descending from the anilino phenol **P-7** which gave expected ethers (e.g. **15**, see Table 1), but reaction with MCPBA produced not only S-oxidation, but in parallel, N-oxidation took place (compounds **22** and **43**). 4-Pyridinol **P-20** afforded nucleophilic substitution products (e.g. **36**, see Table 1).[10] In the case of R1=propylamine oxidation with MCPBA delivered expected compound **56** according to LC-MS analytics whereas in the case of R1=4-fluoroaniline oxidation produced a mixture **35** of variable products which were not elucidated in detail.

Yields of the crude products were averaged over all samples synthesised and exceeded a recovery rate of 80% with respect to the theoretical loading. In vitro testing[11] against IL-8 receptor of project subset **16**–**35** identified highly potent compounds. Selected substances were further purified by reversed phase HPLC and characterised by ^{1}H NMR. Thus, we could confirm samples with high antagonistic potential against IL-8. Table 2 shows IC_{50} values of potent derivatives descending from phenols **P-4** and **P-5** as compared to unsubstituted phenol **P-11**.

Table 1. Result of first library. Selected examples of **1** (n=0) and results of oxidised products **1** (n=2) are shown

	R^1	Ar^1	n	Purity[a]		R^1	Ar^1	n	Purity[a]
15	4-F-$C_6H_4NH_2$	**P-7**	0	91	**36**	n-C_3H_7	**P-20**	0	84
16	4-F-$C_6H_4NH_2$	**P-1**	2	100	**37**	n-C_3H_7	**P-1**	2	100
17	4-F-$C_6H_4NH_2$	**P-2**	2	93	**38**	n-C_3H_7	**P-2**	2	100
18	4-F-$C_6H_4NH_2$	**P-3**	2	68	**39**	n-C_3H_7	**P-3**	2	92
19	4-F-$C_6H_4NH_2$	**P-4**	2	100	**40**	n-C_3H_7	**P-4**	2	100
20	4-F-$C_6H_4NH_2$	**P-5**	2	100	**41**	n-C_3H_7	**P-5**	2	100
21	4-F-$C_6H_4NH_2$	**P-6**	2	91	**42**	n-C_3H_7	**P-6**	2	100
22	4-F-$C_6H_4NH_2$	**P-7**	2	78[b]	**43**	n-C_3H_7	**P-7**	2	88[b]
23	4-F-$C_6H_4NH_2$	**P-8**	2	100	**44**	n-C_3H_7	**P-8**	2	100
24	4-F-$C_6H_4NH_2$	**P-9**	2	63	**45**	n-C_3H_7	**P-9**	2	94
25	4-F-$C_6H_4NH_2$	**P-10**	2	83	**46**	n-C_3H_7	**P-10**	2	88
26	4-F-$C_6H_4NH_2$	**P-11**	2	100	**47**	n-C_3H_7	**P-11**	2	100
27	4-F-$C_6H_4NH_2$	**P-12**	2	94	**48**	n-C_3H_7	**P-12**	2	100
28	4-F-$C_6H_4NH_2$	**P-13**	2	64	**49**	n-C_3H_7	**P-13**	2	82
29	4-F-$C_6H_4NH_2$	**P-14**	2	89	**50**	n-C_3H_7	**P-14**	2	100
30	4-F-$C_6H_4NH_2$	**P-15**	2	69	**51**	n-C_3H_7	**P-15**	2	100
31	4-F-$C_6H_4NH_2$	**P-16**	2	71	**52**	n-C_3H_7	**P-16**	2	100
32	4-F-$C_6H_4NH_2$	**P-17**	2	93	**53**	n-C_3H_7	**P-17**	2	100
33	4-F-$C_6H_4NH_2$	**P-18**	2	84	**54**	n-C_3H_7	**P-18**	2	100
34	4-F-$C_6H_4NH_2$	**P-19**	2	88	**55**	n-C_3H_7	**P-19**	2	100
35	4-F-$C_6H_4NH_2$	**P-20**	2	Low	**56**	n-C_3H_7	**P-20**	2	85

[a] Detected by LC-MS with UV-detection at 210 nM.
[b] According to LC-MS analytics, fully S- and N-oxidised product was isolated.

Table 2. Potency of selected compounds in IL-8 inhibition assay

	Ar^1	IC_{50}/IL-8 (nM)
26	**P-11**	4000
20	**P-5**	250
19	**P-4**	160

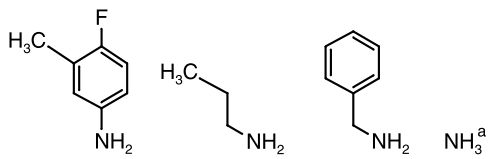


Figure 5. Amines used for the second library. [a]Rink-NH2 resin was used as substitute for ammonia.

With these results in hand, we synthesised a new library, using **3** for the project library part and further diversifying the amide part for general screening. Additionally to **3**, **A-1** and **A-2** which were reductively alkylated to aldehyde resin, Rink-amide resin was used as an ammonia equivalent to evaluate primary amides (**1**, R^1=H) (Fig. 5).

Concerning the ether part Ar^1 of scaffold **1**, expanding the sequence to alkyl ethers was not successful since substitution of the sulfone group with alcoholates under variable conditions did not succeed. Incorporation of a set of substituted phenols with additional functional groups like alcohols (**P-26**) and sulfonic acid amides (**P-29**) and carboxylic acids (**P-21**, **P-23**) showed that these are fully tolerated in the synthesis (Fig. 6). Another 80 examples of general formula **1** were synthesised according to the general protocol. Results of final, oxidised products are shown in Table 3. However, potency of the compounds in the first library for IL-8 was not surpassed anymore.

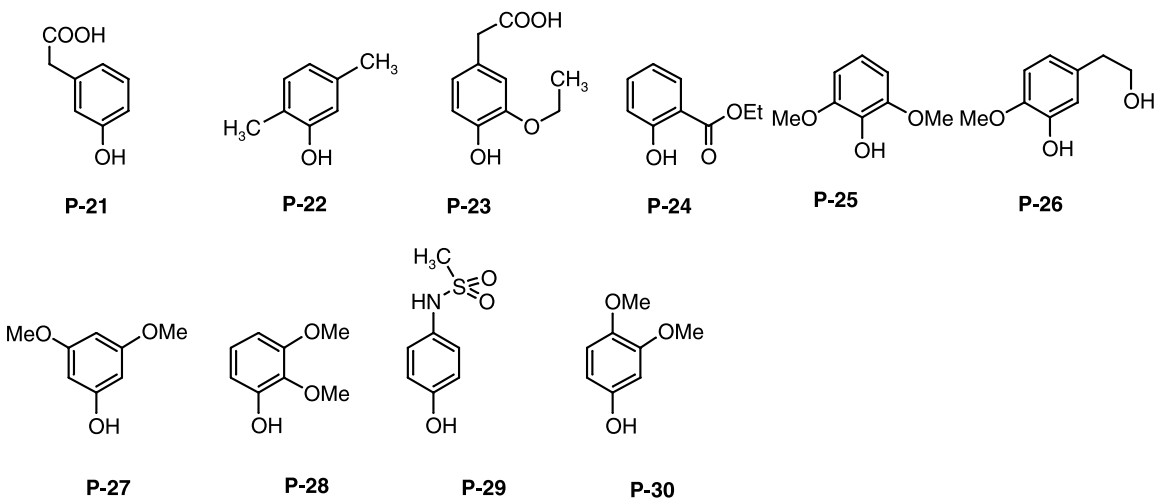


Figure 6. Phenols used in the second library.

Table 3. Result of second library, compounds **1** (n=2) are shown

	R^1	Ar^1	n	Purity[a]		R^1	Ar^1	n	Purity[a]
57	**3**	**P-21**	2	81	**77**	**A-2**	**P-21**	2	93
58	**3**	**P-22**	2	74	**78**	**A-2**	**P-22**	2	100
59	**3**	**P-23**	2	93	**79**	**A-2**	**P-23**	2	92
60	**3**	**P-24**	2	71	**80**	**A-2**	**P-24**	2	94
61	**3**	**P-25**	2	84	**81**	**A-2**	**P-25**	2	89
62	**3**	**P-26**	2	83	**82**	**A-2**	**P-26**	2	100
63	**3**	**P-27**	2	88	**83**	**A-2**	**P-27**	2	94
64	**3**	**P-28**	2	92	**84**	**A-2**	**P-28**	2	100
65	**3**	**P-29**	2	75	**85**	**A-2**	**P-29**	2	91
66	**3**	**P-30**	2	70	**86**	**A-2**	**P-30**	2	100
67	**A-1**	**P-21**	2	93	**87**	**A-3**	**P-21**	2	84
68	**A-1**	**P-22**	2	100	**88**	**A-3**	**P-22**	2	100
69	**A-1**	**P-23**	2	87	**89**	**A-3**	**P-23**	2	80
70	**A-1**	**P-24**	2	76	**90**	**A-3**	**P-24**	2	78
71	**A-1**	**P-25**	2	95	**91**	**A-3**	**P-25**	2	88
72	**A-1**	**P-26**	2	100	**92**	**A-3**	**P-26**	2	100
73	**A-1**	**P-27**	2	100	**93**	**A-3**	**P-27**	2	100
74	**A-1**	**P-28**	2	100	**94**	**A-3**	**P-28**	2	100
75	**A-1**	**P-29**	2	92	**95**	**A-3**	**P-29**	2	86
76	**A-1**	**P-30**	2	100	**96**	**A-3**	**P-30**	2	91

[a] Detected by LC-MS with UV-detection at 210 nM.

3. Conclusion

In summary, we have developed a reliable route to substituted thiadiazolyl ethers using solid phase chemistry. A broad range of phenols was applicable in synthesis procedure. Despite of the multistep synthesis sequence products were cleaved from solid phase in good purity showing the strengths of solid phase synthesis. Furthermore we showed a strategy to combine the advantages of focused and of random combinatorial libraries for pharmaceutical research which can help to further integrate solid phase chemistry into current medicinal chemistry projects.

4. Experimental

4.1. General

Aldehyde resin (0.7–0.9 mmol/g) was obtained from NovaBiochem. Rink-NHFmoc resin was purchased from Rapp Polymere, Tübingen, Germany. Solvents were analytical grade. Reactions were carried out in IRORI Mini Kans. Solvent evaporation was performed on a GeneVac HT-8 cetrifugal evaporator. LC-MS analytics was carried out on a Micromass TOF with MUX-Interface and Waters 600 HPLC; column: symmetry C18, 50 mm×2.1 mm, 3.5 μm; eluent A: acetonitril+0.1% formic acid, eluent B: water+0.1% formic acid; Gradient: 0.0 min 10% A→0.5 min 10% A→4.0 min 90% A→5.5 min 90% A; oven: room temperature, flow: 0.75 ml/min, UV-detection: 210 nm. MS analytics were carried out on a Sciex API 150 with electron spray ionisation. ^{1}H NMR analytics were carried out on a Bruker DPX-300 in $CDCl_3$ as solvent unless otherwise indicated.

During evaluation phase, the course of all reactions was monitored by analytical RP-HPLC and intermediates were checked by LC-MS to ensure structure of the final products. Additionally, selected final compounds were purified by preparative HPLC with a acetonitrile/water-gradient and characterised by ^{1}H NMR.

4.1.1. 2,5-Bis(methylsulfonyl)-1,3,4-thiadiazole (8). 2,5-Bis-methylsulfanyl-[1,3,4]thiadiazole[12] (9.08 g, 50.9 mmol) was suspended in acetic acid (100 ml) and stirred at 60 °C. Hydrogen peroxide (30% (w/w), 255 mmol, 26.0 ml) was added slowly during 1 h. This reaction mixture was reacted for further 5 h at 60 °C and over night at room temperature. The mixture was diluted with water (100 ml), the crystalline product was filtered, washed with water and thereafter with diethyl ether and dried. Yield: 10.2 g (83%). ^{1}H NMR: δ=3.52 (s, 6H).

4.2. Library synthesis

Each 100 mg of 4-(4-formyl-3-methoxyphenoxy)butyryl aminomethyl resin (aldehyde resin, 0.084 mmol/Kan) and a batch of Rink-NHFmoc resin (0.084 mmol/Kan) were dispensed in IRORI-MiniKan reactors. Reactions took place in these reactors. Solvent amounts were adjusted to a minimum, which allows the Kans soaking and free floating. After each reaction step the reactors were washed according to a standard procedure: three times each with DMF, methanol, dichloromethane and diethylether and dried in vacuo unless indicated otherwise. Rink-NHFmoc resin was suspended in piperidine/DMF (1:4), reacted for 20 min at room temperature, filtered and washed according to the standard procedure yielding Rink-NH_2 resin.

4.3. General procedure for reductive alkylation

Aldehyde resin **9** was reacted with primary amines (5 equiv.) over night at 65 °C in toluene/TMOF (4:1). The resin was washed two times with DMF, suspended in DMF and reacted with tetrabutylammonium borohydride (2 equiv.) for 20 min. The reaction was cooled to −40 °C and acetic acid (100 equiv.) was added. The mixture was warmed to room temperature and stirred until development of hydrogen has ceased (appx. 1 h). The resin was washed

first with DMF/diisopropylethylamine and thereafter according to the standard procedure.

In all following reactions, Kans starting with Rink-NH_2 resin and Kans starting with derivatised aldehyde resin, were treated identically.

4.4. General procedure for acylation with 4-chloromethyl benzoic acid chloride

A suspension of diisoproylethylamine (15 equiv.) and 4-chloromethyl benzoic acid chloride (5 equiv.) in dichloromethane was added to resin **10** and stirred for 2 h. The resin **11** was washed with methanol and thereafter according to the standard protocol.

4.5. General procedure for formation of solid phase bound thiol

Resin **11** was suspended in dioxane. Thiourea (8 equiv.) was added and the reaction was stirred over night at 65 °C. The resin was washed according to the standard protocol and suspended in dioxane/water (10:1). Ethylene diamine (10 equiv.) was added and the reaction mixture was stirred at 65 °C for 3 h. Resin **12** was washed according to the standard protocol and immediately used thereafter.

4.6. General procedure for first nucleophilic substitution

DMAP (1.5 equiv.) was reacted for 2 h with a mixture of resin **12** and 2,5-bis(methylsulfonyl)-1,3,4-thiadiazole **8** (1.5 equiv.) in DMF at room temperature. Resin **13** was washed, again.

4.7. General procedure for nucleophilic substitution with phenols

A suspension of resin **13** in dimethyl acetamide was reacted with phenols (10 equiv.) and Cs_2CO_3 (4 equiv.) at room temperature for 6 h. The resin **14** was washed with water and thereafter according to the standard protocol.

4.8. General procedure for thioether oxidation

The resin **14** was suspended in dioxane/2-propanol (3:1). *m*-Chloroperbenzoic acid (10 equiv.) was added and the mixture was reacted for 5 h at room temperature. Resin was washed according to the standard protocol.

4.9. General procedure for cleavage from solid phase

Resins were soaked in TFA four times for 15 min and filtered after each circle. The combined filtrates were evaporated to dryness in vacuo.

Selected samples were further purified by HPLC with a water/acetonitrile-gradient.

4.10. Representative analytic data

4.10.1. N-(4-Fluorophenyl)-4-({[5-(1-naphthyloxy)-1,3,4-thiadiazol-2-yl]sulfonyl}methyl)benzamide (19). ^{1}H NMR: δ=7.95–7.78 (m, 5H), 7.68 (s, 1H), 7.64–7.38 (m, 8H), 7.15–7.04 (m, 2H), 4.8 (d, 2H). MS (ESI+); *m*/*z* (%): 520 [M^++1] (40), 409 (35), 321 (100).

4.10.2. 4-({[5-(2-Ethoxyphenoxy)-1,3,4-thiadiazol-2-yl]sulfonyl}methyl)-N-(4-fluorophenyl)benzamide (20). ^{1}H NMR: δ=7.84 (d, 2H, *J*=8.3 Hz); 7.73 (br s, 1H); 7.55–7.62 (m; 2H); 7.47 (d, 2H, *J*=8.3 Hz,); 7.33–7.22 (m, 2H); 7.13–6.95 (m, 4H); 4.80 (s, 2H); 4.05 (q, 2H, *J*=7.0 Hz); 1.26 (t, 3H, *J*=7.0 Hz). MS (ESI+); *m*/*z* (%): 514 [M^++1] (100), 338 (25).

4.10.3. N-(4-Fluorophenyl)-4-{[(5-phenoxy-1,3,4-thiadiazol-2-yl)sulfonyl]methyl}benzamide (26). ^{1}H NMR (DMSO, 300 MHz): δ=10.32 (s, 1H); 7.85 (d, 2H, *J*=8.3 Hz), 7.84–7.75 (m, 2H); 7.60–7.38 (m, 7H); 7.24–7.15 (m, 2H), 5.20 (s, 2H). MS (ESI+); *m*/*z* (%): 470 [M^++1] (25), 359 (100).

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Rapid microwave-assisted solution phase synthesis of substituted 2-pyridone libraries

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Dedicated to Professor Peter Stanetty on the occasion of his 60th birthday

Abstract—2-Pyridone and 2-quinolone analogues are well-known biologically active heterocyclic scaffolds. Libraries of 3,5,6-substituted 2-pyridone derivatives are generated by rapid microwave assisted solution phase methods using a one-pot, two-step protocol. The three-component condensation of CH–acidic carbonyl compounds, *N*,*N*-dimethylformamide dimethylacetal and methylene active nitriles, leads to 2-pyridones and fused analogues in moderate to good overall yields and high purities. The proposed mechanism of this novel multi-component reaction, structure elucidation of products and intermediates are discussed.

1. Introduction

Many naturally occurring and synthetic compounds containing the 2-pyridone[1] or 2-quinolone[2–4] scaffold possess interesting pharmacological properties. Pyridone **1**, for example, has been identified as specific non-nucleoside reverse transcriptase inhibitor of human immunodeficiency virus-1 (HIV-1).[5,6] Milrinone (**2**), Amrinone (**3**)[7] and their analogues[8–10] are cardiotonic agents for the treatment of heart failure. Some 2-pyridones are also reported to possess antitumor,[11,12] antibacterial[13] and other biological activities.[14–16]

In this work we aimed to develop a rapid microwave-assisted protocol for the solution phase synthesis of highly substituted 2-pyridone derivatives (including fused analogues) which could be applied to a high-throughput format. In view of the structures of several known drugs, we have specified our target 2-pyridone molecules to be unsubstituted in both N1 and C4 positions (Chart 1).

Several synthetic approaches to 2-pyridones of this type are described in the literature. Some of them include rearrangement from a pyrimidine heterocycle,[17–19] others involve ring-opening of 3-formyl-4-hydroxycoumarine[20] and 3-formyl-[21] or 3-cyano-4-oxo-4*H*-1-benzopyran[22] derivatives by the action of enamines or malonodiamide. On the other hand, many literature sources[23–31] describe more general approaches involving the condensation of unsaturated ketones **4** with methylene active amides **5** (Scheme 1). Using cyanoacetamide, a number of Milrinone analogues **7** have been obtained.[24–31]

A few reports[31–33] describe the use of malonodinitrile instead of cyanoacetamide in condensations with enones **4** leading to 3-cyano-2-pyridones **7**. Alternatively, it has been shown that Milrinone can be obtained by the condensation of ethoxymethylene malonodinitrile with 1-(4-pyridyl)-2-propanone **8**.[31] In all cases, strong basic catalysts such as sodium hydride or alkoxides were required for this transformation. However, in earlier work by Junek and

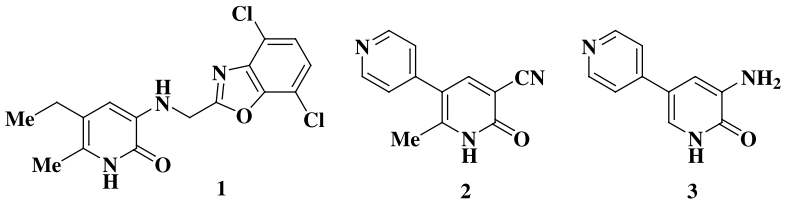

Chart 1.

Keywords: Microwave synthesis; Multi-component reactions; 2-Pyridones; 2-Quinolones.
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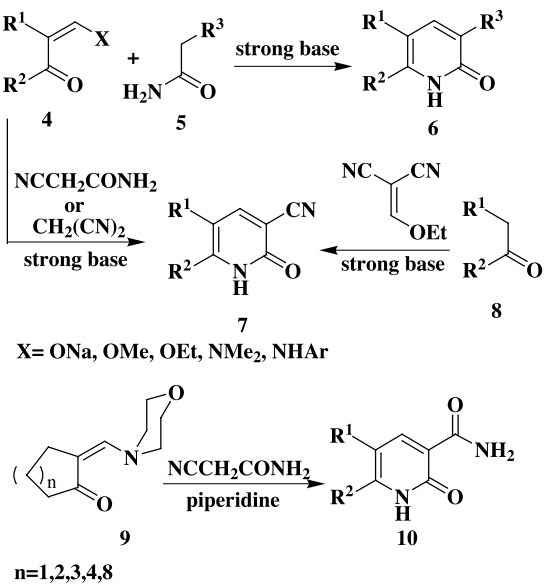


Scheme 1.

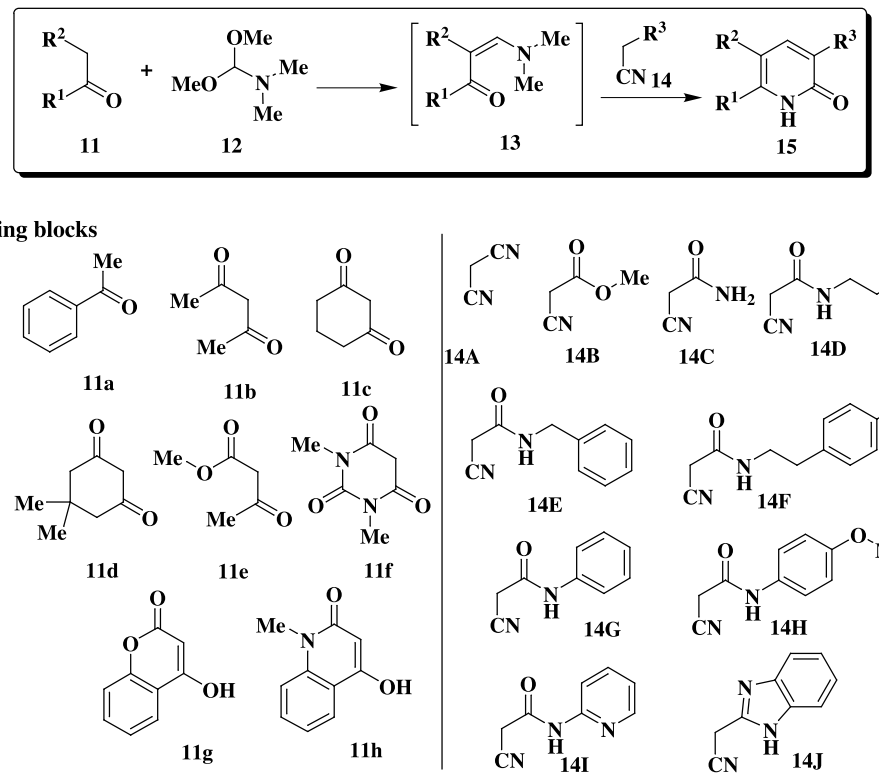
co-workers,[34] it has been shown that the reaction between enamines **9** and cyanoacetamide catalyzed by piperidine readily leads to the desired pyridine-3-carboxamides **10**.

Herein, we introduce general microwave-assisted[35] three-component one-pot synthesis of highly substituted 2-pyridones of type **15**, utilizing CH–acidic substrates **11a–h**, dimethylformamide dimethylacetal (DMFDMA) **12** and diverse methylene active nitriles **14A–J** as building blocks (Scheme 2).[36]

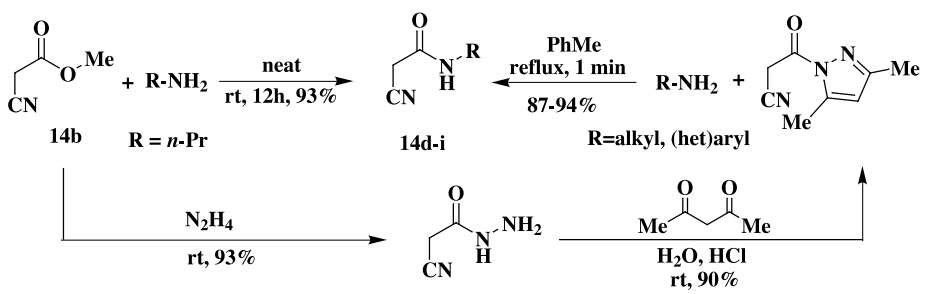
2. Results and discussion

2.1. Building blocks and their preparation

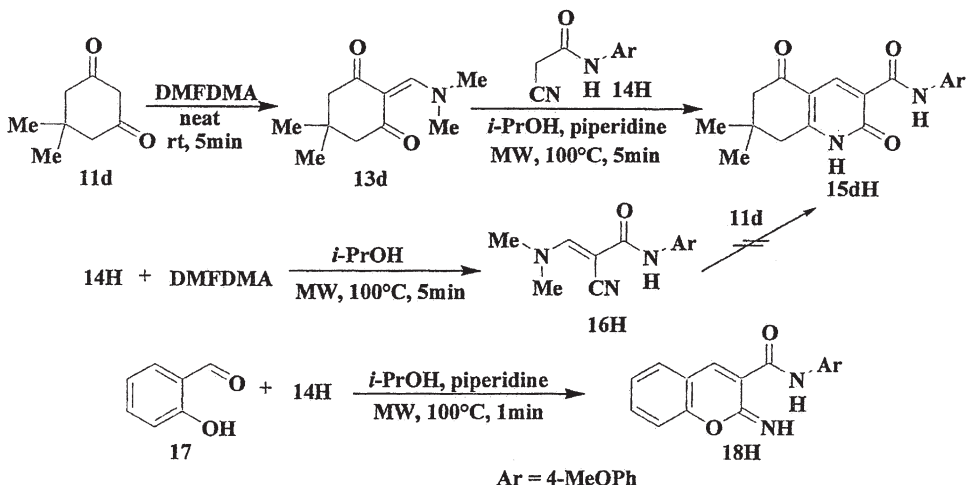
All CH–acidic building blocks **11a–h**, DMFDMA **12** and methylene active nitriles **14A–C**, **14J** were obtained from commercial sources (Scheme 2). 2-Cyano-*N*-propyl-acetamide **14D** was obtained by direct condensation of the methyl cyanoacetate **14B** and *n*-propylamine at room



Scheme 2.



Scheme 3.



Scheme 4.

temperature. The other representatives of various cyanoacetylated aliphatic, aromatic and heterocyclic amines **14E–I** were prepared by trans-cyanoacetylation of the corresponding amines with 1-cyanoacetyl-3,5-dimethylpyrazole in refluxing toluene following known strategies (Scheme 3).[37]

2.2. Optimization of reaction conditions

For comparison purposes we have initially looked at the formation of 2-iminocoumarin derivatives from salicylaldehydes. It is well known that salicylaldehyde derivatives react with methylene active nitriles of type **14** under Knoevenagel reaction conditions (alcoholic media, weak base) to give 2-iminocoumarine derivatives.[38,39] In our reactions, we have considered a similar approach under microwave conditions (MW) (see Scheme 4).

As representative building blocks for CH–acidic compounds **11** and methylene active nitriles **14** (Scheme 2), dimedone **11d** and cyanoacetylated *p*-anisidine **14H** were chosen as starting components. For the first reaction step leading to enamine **13d** (See Scheme 4), initially, we have applied an excess of DMFDMA (1.2 equiv.) at room temperature for 1 min.[40] However, we found that excess of unreacted DMFDMA would effectively react with the third building block, that is, **14H**, to form an adduct **16H**, which would not undergo the desired condensation with dimedone (**11d**). Therefore, it was necessary to carry out this three-component condensation in a two-step fashion and to avoid excess of DMFDMA.

The second step of the two-step sequence (**13**+**14**→**15**) was optimized by varying reaction time and temperature under microwave irradiation conditions, and using different solvents (MeOH, EtOH, *i*-PrOH) or solvent-free conditions. The best result with respect to isolated yields and purities of the final compound was obtained when a mixture of adduct **13d**, nitrile **14H** and catalytic amounts of piperidine in *i*-PrOH was irradiated at 100 °C for 5 min. Upon cooling, product **15dH** could be isolated by simple filtration in moderate yield (45%) but excellent purity (>98%). In a comparison experiment with salicylaldehyde **17** and nitrile **14H**, 1 min of microwave irradiation at 100 °C gave the corresponding 2-iminocoumarin **18H** in virtually quantitative yield (Scheme 4).[41]

2.3. Library preparation

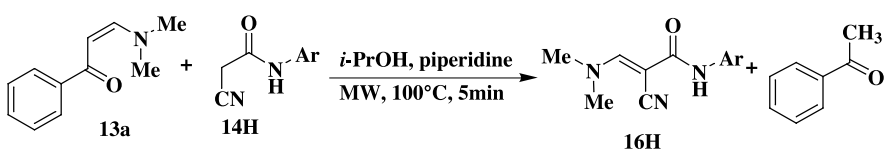
Optimized conditions for the synthesis of the key enamine intermediates **13** are shown in Table 1. In case of 1,3-dicarbonyl compounds **11c**,**d**, the initial condensation with DMFDMA was completed within 5 min at room temperature. For other building blocks the reaction took place at elevated-temperature microwave conditions (Table 1).

Table 1. Enamine **13a–h** preparation under solvent-free conditions

Reagents[a]	Adduct	Time (min)	Temp[b] (°C)	Conversion[c] (%)
11a+12	**13a**	10	170	95
11b+12	**13b**	5	100	>99
11c+12	**13c**	5	rt	>99
11d+12	**13d**	5	rt	>99
11e+12	**13e**	5	100	>99
11f+12	**13f**	5	100	>99
11g+12	**13g**	5	150	69[d]
11h+12	**13h**	5	150	68[d]

[a] Equimolar amounts (2 mmol each).
[b] Microwave irradiation (single-mode, for details see Section 4).
[c] Determined by RP-HPLC.
[d] Isolated yield of compound.

Initially, the transformation of adducts **13a–h** to 2-pyridones **15** was studied using model nitrile **14H** with the previously optimized conditions (MW, 100 °C, 5 min). While compounds **15bH–15eH** were isolated in good yield,



Scheme 5.

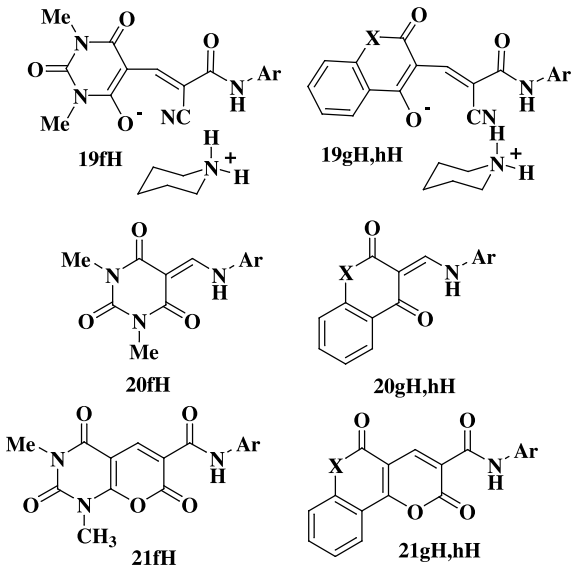


Chart 2.

there were particular cases that showed the limitations of this protocol. Thus, the reaction between acetophenone adduct **13a** and nitrile **14H** unexpectedly led to cyano-enamine **16H** and acetophenone (Scheme 5).

In cases of 1,3-dimethylbarbituric acid **11f**, 4-hydroxy-coumarine **11g** and 4-hydroxy-1-methyl-1*H*-quinolin-2-one **11h**, it was found that the catalyst (piperidine) was selectively consumed from the reaction medium leading to complex mixtures. When 1.0 equiv. of piperidine was used in these transformations the stoichiometric salts **19fH**, **gH**, **hH** were formed as crystalline solids. When the reaction temperature was increased to 150 °C to force cyclization, only the undesired enamines **20fH**, **gH**, **hH** were obtained. Adding concentrated aqueous HCl to the final reaction mixtures or to isolated salts **19fH**, **gH**, **hH** in *i*-PrOH and irradiating again at 120 °C for 2 min furnished the corresponding pyran derivatives **21fH**, **gH**, **hH**. Only in the case of 1,3-dimethylbarbituric acid **11f** and 4-hydroxy-1-methyl-1*H*-quinolin-2-one **11h** the desired 2-pyridones **15fH** and **15hH** were obtained by microwave irradiation of the isolated salt **19fH** and **19hH** in *i*-PrOH at 150 °C for 10 min, respectively (Chart 2).

In order to synthesize all the desired 2-pyridone library compounds **15**, methylene active nitriles **14A**–**J** were employed in condensations with cyclohexanedione **13c** and dimedone **13d** enamine adducts. In cases of compounds **15cC**, **cD**, **cE**, **cG**, **cH**, **cI**, **dA**, **dB**, **dC**, **dE**, **dF** (Table 2), the desired 2-pyridone products were formed. Surprisingly, however, cyclohexanedione **13c** and dimedone **13d** enamine adducts showed somewhat different reactivity toward cyanoacetoamide **14C** and 2-cyano-*N*-pyridin-2-yl-acetamide **14I**. Under the applied conditions in case of cyclohexanedione **13c**, the expected 2-pyridones **15cC** and **15cI** were formed. On the other hand, dimedone adduct **13d** gave the open chain salts **19dC** and **19dI** which produced the desired pyridone products **15dC** and **15dI** only by irradiation at higher temperature (Scheme 6). Interestingly, applying a solvent-free protocol (100 °C, 5 min), products **15dC** and **15dI** were obtained directly from starting materials **13d**, **14C** and **14I**. Similarly, under solvent free conditions (100 °C, 5 min), 3-carbomethoxy-2-pyridone **15dB** was obtained without any open chain impurity.

Table 2. Conditions and yields for synthesized compounds

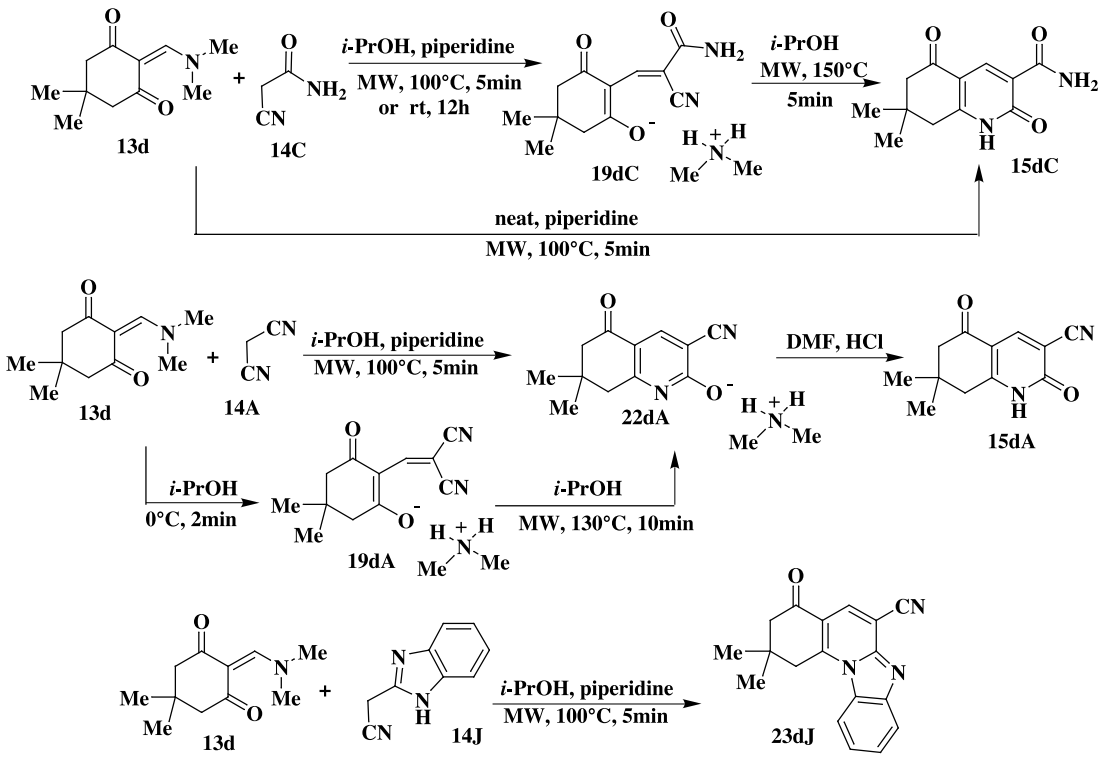
Compound	Scale[a] (mmol)	Temp (°C)	Time (min)	Yield[b] (%)
15bH	4	100	5	67
15cC	2	100	5	78
15cD	4	100	5	72
15cG	2	100	5	81
15cH	2	100	5	55
15cI	2	100	5	54
15dA	0.38	25	5	85[c]
15dB[d]	4	100	5	53
15dC	4	100	5	43[d]
15dD	4	100	5	31
15dE	4	100	5	55
15dF	4	100	5	58
15dG	4	100	5	27
15dH	4	100	5	55
15dI	1	150	5	57[d]
15eH	4	100	5	65
15fH[d]	1.24	150	10	70
15hH[d]	2	150	10	79
16H	1	100	5	65
18H	2	100	1	96
19dA	4	0	2	64
19dC	4	25	12	78
19dI	4	100	5	68
19fH	2	100	5	68
19gH	2	100	5	89
19hH	2	100	5	75
20fH	2	150	5	55
20gH	2	150	5	48
20hH	2	150	5	58
21fH	2	120	2	53
21gH	2	120	2	58
21hH	2	120	2	56
22dA	4	100	5	55
23dJ	2	100	5	78
24dC	0.5	130	3	47

[a] Equimolar amounts of **11**, **12** and **14** in *i*-PrOH (2 mL).
[b] Isolated yield of pure (>98% by HPLC) compound.
[c] Starting from **22dA**.
[d] See text for details.

Utilizing malonodinitrile **14A** as building block, the initially formed salt **22dA** was extraordinarily stable and was isolated both directly from **13d** and **14A** (100 °C, 5 min) and also via irradiation of intermediate salt **19dA** under microwave conditions (130 °C, 10 min) (Scheme 6). Another unusual result was obtained when 2-cyanomethyl-benzimidazole **14J** was utilized as building block. In this case, cyclization involved the imidazole ring to furnish tetracyclic product **23dJ** (Scheme 6).

2.4. Structure elucidation

In the course of this work different spectroscopic methods for structure elucidation of all intermediates and products have been used. Here some model cases are discussed. Full spectral data for all new compounds are presented in the Section 4. The distinction of structure **15dH** from the possible isomeric structures 2-iminopyran **15*** and the open-chain unsaturated hydroxy nitrile **15*** * was performed by NOE NMR experiments. Irradiation at 2.84 ppm (C8–H) produced a distinct NOE effect at 13.09 ppm (NH). Furthermore, full assignment of the ^{13}C NMR spectrum



Scheme 6.

by performing HMBC experiments confirmed structure **15dH**, where the key signal at δ 161.7 ppm was assigned to the lactam carbonyl group. In the solid phase IR spectra of compounds **15** no nitrile absorption band was present. For product **23dJ** two isomeric structures (**23dJ**, **23dJ***) can be proposed. HMBC experiments have confirmed a correlation between the nitrile carbon and the alkene proton in the pyridine ring, which is possible only for structure **23dJ** (Chart 3).

The structure of salt **22dA** was determined by X-ray crystallography (Fig. 1)[42] in addition to a full spectroscopic analysis.

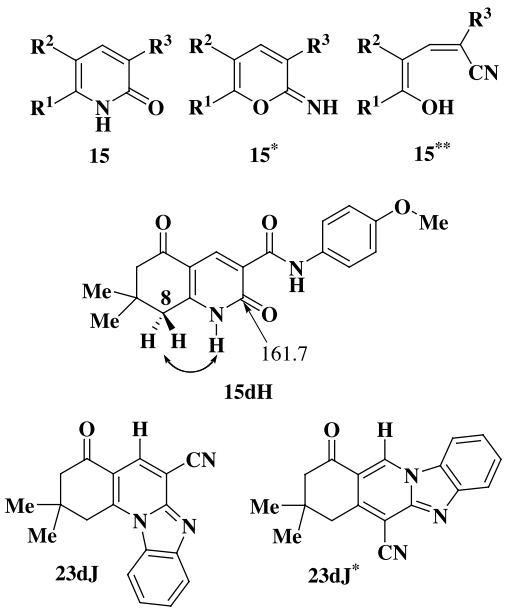


Chart 3.

To confirm the structure of the open-chain salt for the example of dimedone derivative **19dC** and also to add another diversity point to the final 2-pyridone libraries the reaction with *p*-anisidine was performed (Scheme 7). The structure of the *N*-arylated product **24dC** was confirmed by all routine spectral data and NOE.

Based on all the above results and the structure elucidation of isolated reaction intermediates we propose the following reaction mechanism for the formation of 2-pyridones (Scheme 8).

After the formation of 2-iminopyrane intermediate **26** the next step is the ring opening with base (**26**→**27**) and subsequent ring-closure to the pyridine in a Dimroth-type rearrangement. In case of 2-iminocoumarine (where R^1/R^2 constitutes a fused benzene cycle, see Scheme 4) this is not possible. In the aliphatic cases, nucleophilic attack on the C-6 position of the 2-iminolactone **26** becomes viable and after Dimroth-like rotation the thermodynamically more stable 2-pyridone **15** is formed.

3. Conclusion

In conclusion, we have demonstrated that libraries of densly substituted 2-pyridones of type **15** can be rapidly prepared by solution-phase methods, employing a one pot, two step protocol, that involves the condensation of CH–acidic carbonyl compounds with *N*,*N*-dimethylformamide dimethylacetal (DMFDMA) and methylene active nitriles. The isolated yields in this microwave-assisted protocols

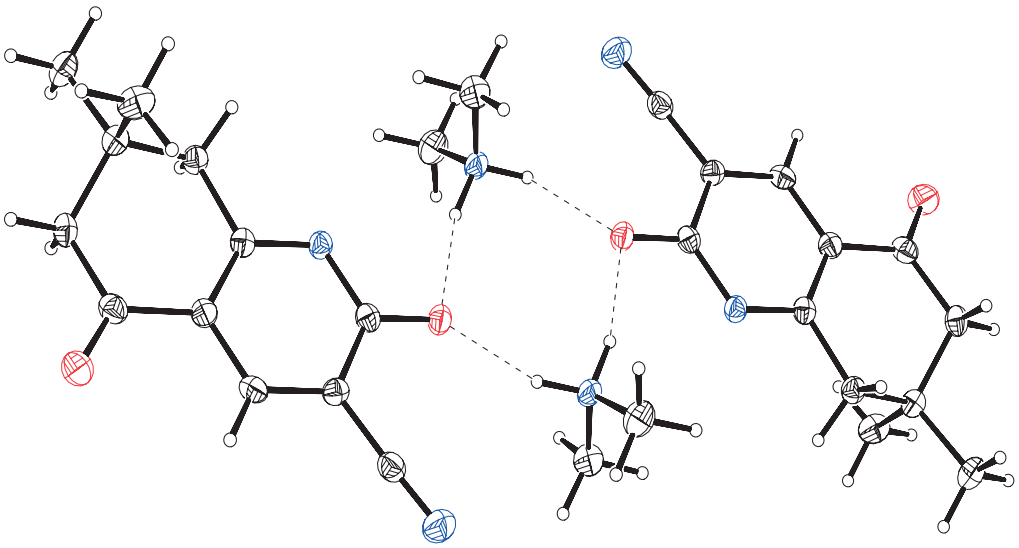
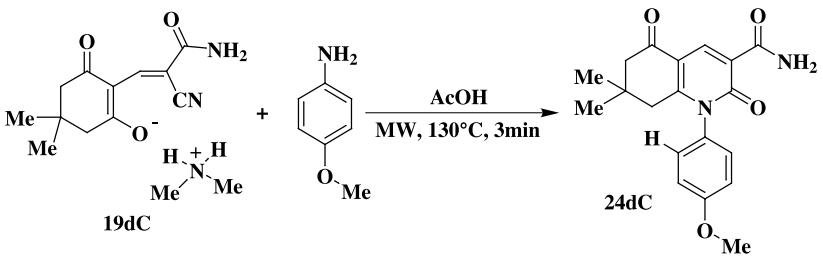
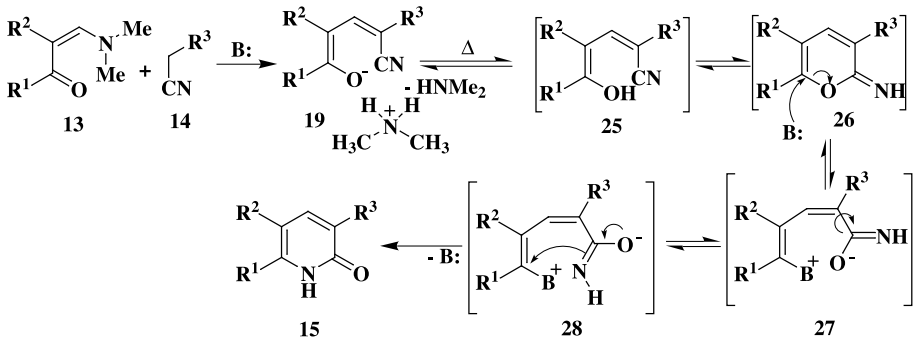


Figure 1. ORTEP plot of the hydrogen bonded salt **22dA**.



Scheme 7.



Scheme 8.

were moderate to high (27–96%) and provided the desired target compounds in high purity after simple workup by filtration.

4. Experimental

Starting materials were obtained from commercial suppliers and used without further purification. TLC analysis was performed on Merck precoated 60 F_{254} plates. Melting points were obtained on a Gallenkamp melting point apparatus, Model MFB-595 in open capillary tubes. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX360 and 500 MHz instruments in $CDCl_3$ or DMSO-d_6. IR spectra were taken on a Perkin–Elmer 298 spectrophotometer in KBr pellets. Mass spectra were taken on a Hewlett–Packard LC/MSD 1100 series instrument in the atmospheric pressure chemical ionization (negative or positive APCI) mode. Micro-analyses were obtained on a Fisons Mod. EA 1108 elemental analyzer. For reaction monitoring, kinetic investigations, and quality (purity) control of the synthesized library compounds a Shimadzu LC-10 system, that included LC10-AT(VP) pumps, an autosampler (S-10AXL), and a dual wavelength UV detector set at 215 and 280 nm was used. The separations were carried out using a C18 reversed phase analytical column, LiChrospher 100 (E. Merck, 100×3 mm, particle size 5 μm) at 25 °C and a mobile phase from (A) 0.1% TFA in 90:10 water/MeCN and (B) 0.1% TFA acid in MeCN (all solvents were HPLC grade, Acros; TFA was analytical

reagent grade, Aldrich). The following gradients were applied at a flow rate of 0.5 mL/min: linear increase from solution 30% B to 100% solution B in 7 min, hold at 100% solution B for 1 min. Sample preparation was done by diluting approximately 5 μL of the MeCN solution with 0.5 mL of MeCN. 5 μL of the solutions were injected onto the HPLC system.

4.1. Microwave irradiation experiments

All microwave irradiation experiments were carried out using the Emrys™ Synthesizer from PersonalChemistry AB (Uppsala). All experiments were performed in sealed microwave process vials utilizing the standard absorbance level (300 W maximum power). Reaction times under microwave conditions reflect the time the reaction mixture was kept at the designated temperature (fixed hold time). A detailed description of this single-mode microwave reactor with integrated robotics was recently published.[43]

4.2. Preparation of *N*1-propyl-2-cyanoacetamide (14D)

An equimolar mixture of *n*-propylamine (2.96 g, 50 mmol) and methyl cyanoacetate **14B** (4.95 g, 50 mmol) was stirred at room temperature overnight. The formed crystalline product was filtered, washed with Et_2O, and dried (5.95 g, yield 94%), mp 48 °C.

4.3. General procedure for the preparation of *N*1-substituted 2-cyanoacetamides (14E–I)[37]

Cyanoacetic acid hydrazide was obtained by careful addition of 19.82 g (0.20 mol) of methyl cyanoacetate to hydrazine hydrate (10.01 g, 0.20 mol) with stirring at room temperature. The formed cyanoacetic acid hydrazide was filtered, washed with Et_2O, and dried (yield 93%). To a stirred solution of the cyanoacetic acid hydrazide (9.91 g, 0.10 mol) in water (25 mL) containing conc. HCl (1 mL) at room temperature, acetylacetone (10.01 g, 0.10 mol) was added. The mixture was stirred for about 1 h, until the precipitation was complete. 1-Cyanoacetyl-3,5-dimethylpyrazole was removed by filtration, washed with water, and dried (mp 118 °C, yield 90%). This product (4.08 g, 25.0 mmol) was added to a solution of 1.0 equiv. of amine derivative in toluene (10–15 mL), the mixture was refluxed for 1 min, and then stirred at room temperature. The *N*-substituted 2-cyanoacetamides (**14E–I**) were filtered, washed with Et_2O and dried (**14E** mp 120 °C, **14F** mp 88 °C, **14G** mp 198 °C, **14H** mp 136 °C, **14I** mp 155 °C; with ca. 90% yields).[37,44]

4.4. General procedure for the synthesis of 2-pyridones 15

An equimolar mixture of carbonyl compound **11b–e** and DMFDMA **12** (2.0/4.0 mmol, see Table 2) in a 2.5 mL (small) Emrys™ microwave process vial was stirred under the conditions specified in Table 1 to form enamines **13b–e**. Subsequently, methylene active nitriles **14A–I** (1.0 equiv.), 2.0 mL of *i*-PrOH (for **15dB** and **15dC** without *i*-PrOH) and piperidine (2 drops, ca. 28 mg) were added to the vial and the mixture was irradiated under microwave conditions at 100 °C for 5 min. After cooling, in most cases a crystalline precipitate was formed. Otherwise, precipitation was initiated by cooling (4 °C). After several hours of stirring the precipitate was filtered, washed with *i*-PrOH (2×1 mL), Et_2O and subsequently dried. Additional amounts of product can be collected from the filtrate after further precipitation overnight. All compounds had >98% purity by HPLC and were identified by mp, NMR, IR, MS and elemental analysis. For yields see Table 2. When appropriate (see below), analytical samples were obtained by re-crystallization from a suitable solvent.

4.4.1. *N*3-(4-Methoxyphenyl)-5-acetyl-6-methyl-2-oxo-1,2-dihydro-3-pyridinecarboxami-de (15bH). Mp 328 °C dec. Anal. Calcd ($C_{16}H_{16}N_2O_4$) C, 63.99; H, 5.37; N, 9.33. Found: C, 64.24; H, 5.40; N, 9.43; ^{1}H NMR (DMSO-d_6) δ 2.51 (s, 3H), 2.60 (s, 3H), 3.74 (s, 3H), 6.92 (d, J=9.0 Hz, 2H), 7.61 (d, J=9.0 Hz, 2H), 8.79 (s, 1H), 11.55 (s, 1H), 13.00 (s, 1H); MS (pos. APCI) *m/z* 301 (M+1); IR (KBr), cm^{-1}: ν 1368, 1511, 1564, 1622, 1679, 1700, 2913, 3000, 3281.

4.4.2. 2,5-Dioxo-1,2,5,6,7,8-hexahydro-3-quinolinecarboxamide (15cC). Mp 330 °C dec. (lit.[45] 340 °C dec.). Anal. Calcd ($C_{10}H_{10}N_2O_3$): C, 58.25; H, 4.89; N, 13.59. Found: C, 57.99; H, 4.84; N, 13.48; ^{1}H NMR (DMSO-d_6) δ 2.03 (quintet, J=6.1 Hz, 2H), 2.48 (t, J=6.7 Hz, 2H), 2.87 (t, J=5.9 Hz, 2H), 7.65 (s, 1H), 8.63 (s, 1H), 8.67 (s, 1H), 12.68 (s, 1H); MS (pos. APCI) *m/z* 207 (M+1); IR (KBr), cm^{-1}: ν 1394, 1559, 1664, 1684, 2958, 3099, 3359, 3500.

4.4.3. *N*3-Propyl-2,5-dioxo-1,2,5,6,7,8-hexahydro-3-quinolinecarboxamide (15cD). Mp 328 °C dec. (recrystallized analytical sample). Anal. Calcd ($C_{13}H_{16}N_2O_3$): C, 62.89; H, 6.50; N, 11.28. Found: C, 63.11; H, 6.45; N, 11.24. ^{1}H NMR (DMSO-d_6) δ 0.88 (t, J=7.4 Hz, 3H), 1.50 (hextet, J=7.2 Hz, 2H), 2.03 (quintet, J=6.2 Hz, 2H), 2.48 (t, J=6.9 Hz, 2H), 2.88 (t, J=6.0 Hz, 2H), 3.25 (quartet, J=6.4 Hz, 2H), 8.63 (s, 1H), 9.35 (s, 1H), 12.83 (s, 1H); MS (pos. APCI) *m/z* 249 (M+1); IR (KBr), cm^{-1}: ν 1398, 1545, 1559, 1661, 1685, 3119.

4.4.4. *N*3-Phenyl-2,5-dioxo-1,2,5,6,7,8-hexahydro-3-quinolinecarboxamide (15cG). Mp 339 °C. Anal. Calcd ($C_{16}H_{14}N_2O_3$): C, 68.07; H, 5.00; N, 9.92. Found: C, 67.88; H, 4.84; N, 9.90; ^{1}H NMR (DMSO-d_6) δ 2.06 (quintet, J=6.1 Hz, 2H), 2.44–2.55 (m, 2H), 2.93 (t, J=5.9 Hz, 2H), 7.13 (t, J=7.4 Hz, 1H), 7.36 (t, J=7.8 Hz, 2H), 7.68 (d, J=7.8 Hz, 2H), 8.74 (s, 1H), 11.64 (s, 1H), 13.09 (s, 1H); MS (pos. APCI) *m/z* 283 (M+1); IR (KBr), cm^{-1}: ν 1394, 1554, 1597, 1627, 1683, 2964.

4.4.5. *N*3-(4-Methoxyphenyl)-2,5-dioxo-1,2,5,6,7,8-hexahydro-3-quinolinecarboxamide (15cH). Mp 323 °C dec. Anal. Calcd ($C_{17}H_{16}N_2O_4$): C, 65.38; H, 5.16; N, 8.97. Found: C, 64.99; H, 5.01; N, 9.28; ^{1}H NMR (DMSO-d_6) δ 2.05 (quintet, J=6.0 Hz, 2H), 2.44–2.55 (m, 2H), 2.92 (t, J=5.9 Hz, 2H), 3.74 (s, 3H), 6.92 (d, J=8.9 Hz, 2H), 7.61 (d, J=8.9 Hz, 2H), 8.72 (s, 1H), 11.50 (s, 1H), 13.00 (br s, 1H); MS (pos. APCI) *m/z* 313 (M+1); IR (KBr), cm^{-1}: ν 1235, 1510, 1558, 1680, 2910, 3154.

4.4.6. *N*3-(2-Pyridyl)-2,5-dioxo-1,2,5,6,7,8-hexahydro-3-quinolinecarboxamide (15cI). Mp 335 °C (recrystallized

analytical sample). Anal. Calcd ($C_{15}H_{13}N_3O_3$): C, 63.60; H, 4.63; N, 14.83. Found: C, 63.72; H, 4.26; N, 14.60; ^{1}H NMR (DMSO-d_6) δ 2.06 (quintet, J=6.1 Hz, 2H), 2.51 (t, J=6.6 Hz 2H), 2.93 (t, J=5.8 Hz, 2H), 7.15 (t, J=6.1 Hz, 1H), 7.83 (t, J=7.8 Hz, 1H), 8.26 (d, J=8.3 Hz, 2H), 8.34 (d, J=4.6 Hz, 1H), 8.77 (s, 1H), 12.04 (s, 1H), 13.12 (br s, 1H); MS (pos. APCI) m/z 284 (M+1); IR (KBr), cm^{-1}: ν 1433, 1530, 1633, 1680, 3245.

4.4.7. 7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydro-3-quinolinecarbonitrile (15dA). The product was obtained by decomposition of salt **22dA** (100 mg, 0.38 mmol) in DMF (0.5 mL) with conc. HCl (0.5 mL) at room temperature. After addition of 1 mL water the mixture was filtered and the product washed with Et_2O (2×1 mL). Yield: 85%, mp 310–311 °C (lit.[45] 298 °C); ^{1}H NMR ($CDCl_3$) δ 1.19 (s, 6H), 2.50 (s, 2H), 2.90 (s, 2H), 8.51 (s, 1H), 12.60 (b s, 1H); IR (KBr), cm^{-1}: ν 955, 1154, 1407, 1597, 1669, 2218, 2785, 2947, 3000, 3055.

4.4.8. Methyl-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydro-3-quinolinecarboxylate (15dB). The compound was obtained as white solid by a solvent free protocol under microwave conditions at 100 °C for 5 min (yield 53%), mp 232 °C. Anal. Calcd ($C_{13}H_{15}NO_4$): C, 62.64; H, 6.07; N, 5.62. Found: C, 62.86; H, 6.07; N 5.56; ^{1}H NMR ($CDCl_3$) δ 1.16 (s, 6H), 2.48 (s, 2H), 2.92 (s, 2H), 3.92 (s, 3H), 8.80 (s, 1H); MS (pos. APCI) m/z 250 (M+1); IR (KBr), cm^{-1}: ν 1395, 1641, 1690, 1744, 2962, 3069, 3213, 3146. The same compound was obtained by a solvent free protocol under microwave conditions at 100 °C for 5 min (yield 53%).

4.4.9. 7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydro-3-quinolinecarboxamide (15dC). The compound was obtained by irradiation of 200 mg (0.7 mmol) of salt **19dC** in *i*-PrOH (1 mL) under microwave conditions at 150 °C for 5 min, yielding 78 mg (48%) of product, mp 346 °C dec. (lit.[45a] 340 °C dec.); ^{1}H NMR (DMSO-d_6) δ 2.39 (s, 2H), 2.79 (s, 2H), 7.67 (s, 1H), 8.62 (s, 1H), 8.66 (s, 1H), 12.74 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 28.1, 33.1, 50.5, 112.8, 119.4, 140.4, 159.2, 163.5, 164.5, 193.7; MS (pos. APCI) m/z 235 (M+1); IR (KBr), cm^{-1}: ν 1407, 1598, 1669, 2874, 2958, 3350. The same compound was obtained as white solid by a solvent free protocol under microwave conditions at 100 °C for 5 min (yield 43%).

4.4.10. *N*3-Propyl-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydro-3-quinolinecarboxamide (15dD). Mp 325 °C dec. (recrystallized analytical sample). Anal. Calcd ($C_{15}H_{20}N_2O_3$): C, 65.20; H, 7.30; N, 10.14. Found: C, 65.15; H, 7.47; N, 10.28. ^{1}H NMR (DMSO-d_6) δ 0.88 (t, J=7.4 Hz, 3H), 1.01 (s, 6H), 1.49 (hextet, J=7.2 Hz, 2H), 2.39 (s, 2H), 2.77 (s, 2H), 3.25 (quartet, J=6.4 Hz, 2H), 8.61 (s, 1H), 9.38 (t, 1H), 12.65 (br s, 1H); MS (pos. APCI) m/z 277 (M+1); IR (KBr), cm^{-1}: ν 1312, 1430, 1551, 1615, 1663, 2795, 2834, 2940, 2963, 3115, 3324.

4.4.11. *N*3-Benzyl-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydro-3-quinolinecarboxamide (15dE). Mp 271–273 °C. Anal. Calcd ($C_{19}H_{20}N_2O_3$) C, 70.35; H, 6.21; N, 8.64. Found: C, 70.55 H, 6.20 N, 8.60; ^{1}H NMR (DMSO-d_6) δ 1.02 (s, 6H), 2.41 (s, 2H), 2.80 (s, 2H), 4.53 (d, J=5.8 Hz, 2H), 7.20–7.44 (m, 5H), 8.66 (s, 1H), 9.72 (t, J=5.8 Hz, 1H), 12.90 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 28.1, 33.1, 42.8, 50.5, 113.0, 118.8, 127.4, 127.8, 128.9, 139.7, 140.3, 159.1, 163.1, 163.6, 193.7; MS (pos. APCI) m/z 325 (M+1); IR (KBr), cm^{-1}: ν 1398, 1570, 1667, 2958, 2958, 3091.

4.4.12. *N*3-(4-Fluorophenethyl)-7,7-dimethyl-2,5-dioxo-1,2,4a,5,6,7,8,8a-octahydro-3-quinolinecarboxamide (15dF). Mp 251 °C. Anal. Calcd ($C_{20}H_{21}FN_2O_3$) C, 67.40; H, 5.94; N, 7.86. Found: C, 67.47; H, 5.86; N, 7.90; ^{1}H NMR (DMSO-d_6) δ 1.01 (s, 6H), 2.39 (s, 2H), 2.77 (s, 2H), 2.80 (t, J=7.2 Hz 2H), 3.52 (q, J=7.2 Hz, 2H), 7.07–7.12 (m, 2H), 7.25–7.29 (m, 2H), 8.61 (s, 1H), 9.36 (s, 1H), 12.80 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 28.1, 33.0, 34.7, 39.2, 40.7, 50.5, 112.9, 115.4 (d, J=20.8 Hz), 118.9, 130.9 (d, J=7.6 Hz), 135.9, 140.0, 158.9, 161.3 (d, J=239.9 Hz), 162.9, 163.5, 193.7; MS (pos. APCI) m/z 357 (M+1); IR (KBr), cm^{-1}: ν 1508, 1557, 1618, 1678, 2361, 3057.

4.4.13. *N*3-Phenyl-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydro-3-quinolinecarboxamide (15dG). Mp 332 °C. Anal. Calcd ($C_{18}H_{18}N_2O_3$): C, 69.66; H, 5.85; N, 9.03. Found: C, 69.87; H, 5.74; N, 9.19; ^{1}H NMR (DMSO-d_6) δ 1.04 (s, 6H), 2.44 (s, 2H), 2.85 (s, 2H), 7.12 (t, J=7.5 Hz, 1H), 7.37 (t, J=7.7 Hz, 2H), 7.69 (d, J=7.9 Hz, 2H), 8.73 (s, 1H), 11.64 (s, 1H); MS (pos. APCI) m/z 311 (M+1); IR (KBr), cm^{-1}: ν 1392, 1484, 1599, 1626, 1683, 2751, 2799, 2876, 2920, 2965, 3012, 3027, 3081.

4.4.14. *N*3-(4-Methoxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydro-3-quinolinecarboxamide (15dH). Mp 300 °C dec. Anal. Calcd ($C_{19}H_{20}N_2O_4$) C, 67.05; H, 5.92; N, 8.23. Found: C, 66.99; H, 5.95; N, 8.43; ^{1}H NMR (DMSO-d_6) δ 1.04 (s, 6H), 2.43 (s, 2H), 2.84 (s, 2H), 3.74 (s, 3H), 6.93 (d, J=9.0 Hz, 2H), 7.61 (d, J=9.0 Hz, 2H), 8.71 (s, 1H), 11.47 (s, 1H), 13.09 (s, 1H); ^{13}C NMR (DMSO-d_6) 27.2 (2Me), 37.4 (CH_2-6), 41.3 (CH_2-8), 50.1 (C-7), 54.4 (OMe), 111.5 (C-4a), 114.2 (CH-3′,5′), 120.3 (C-3), 122.5 (CH-2′,6′), 131.2 (C-1′), 139.1 (CH-4), 154.3 (C-4′), 158.6 (C-8a), 160.4 (CONHAr), 161.7 (CO-2), 180.2 (CO-5); MS (pos. APCI) m/z 341 (M+1); IR (KBr), cm^{-1}: ν 1511, 1556, 1596, 1630, 1675, 2966.

4.4.15. *N*3-(2-Pyridyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydro-3-quinolinecarbox-amide (15dI). The compound was obtained by irradiation of 356 mg (1 mmol) of salt **19dI** in *i*-PrOH (2 mL) under microwave conditions at 150 °C for 5 min, yielding 178 mg (57%) of product; mp 328 °C dec. Anal. Calcd ($C_{17}H_{17}N_3O_3$): C, 65.58; H, 5.50; N, 13.50. Found: C, 65.71; H, 5.26; N, 13.69. ^{1}H NMR (DMSO-d_6) δ 1.04 (s, 6H), 2.44 (s, 2H), 2.85 (s, 2H), 7.16 (t, J=6.0 Hz, 1H), 7.65 (d, J=7.7 Hz, 1H), 7.84 (t, J=6.7 Hz, 1H), 8.26 (d, J=7.8 Hz, 1H), 8.76 (s, 1H), 12.02 (s, 1H), 13.32 (br s, 1H); MS (pos. APCI) m/z 312 (M+1); IR (KBr), cm^{-1}: ν. 1492, 1540, 1573, 1633, 1681, 2756, 2792, 2839, 2869, 2896, 2935, 2955, 3018.

4.4.16. Methyl 3-(4-methoxyphenylcarbamoyl)-6-methyl-2-oxo-1,2-dihydro-5-pyridinecarboxylate (15eH). Mp 326 °C dec. Anal. Calcd ($C_{16}H_{16}N_2O_5$) C,

60.75; H, 5.10; N, 8.86. Found: C, 60.87; H, 5.22; N, 8.90; ^{1}H NMR (DMSO-d_6) δ 2.65 (s, 3H), 3.74 (s, 3H), 3.81 (s, 3H), 6.93 (d, J=9.0 Hz, 2H), 7.61 (d, J=9.0 Hz, 2H), 8.79 (s, 1H), 11.53 (s, 1H), 13.04 (br s, 1H); MS (pos. APCI) m/z 317 (M+1); IR (KBr), cm^{-1}: ν 1374, 1509, 1559, 1682, 1715, 2913, 3000, 3127.

4.4.17. *N*-(4-Methoxyphenyl)-1,3-dimethyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydro-pyrido[2,3-*d*]pyrimidine-6-carboxamide (15fH). The title compound was obtained as pale yellow solid by microwave irradiation of 546 mg (1.24 mmol) of salt **19fH** in *i*-PrOH (2 mL) at 150 °C for 10 min, yield 312 mg (70%), mp 334 °C dec. Anal. Calcd ($C_{17}H_{16}N_4O_5$): C, 57.30; H, 4.53; N, 15.72. Found: C, 57.18; H, 4.36; N, 15.58; ^{1}H NMR (DMSO-d_6) δ 2.49 (s, 6H), 3.72 (s, 3H), 4.19 (br, s, 2H), 6.86 (d, J=9.0 Hz, 2H), 7.53 (d, J=9.0 Hz, 2H), 8.25 (s, 1H), 8.91 (s, 1H); MS (pos. APCI) m/z 356; IR (KBr), cm^{-1}: ν 1290, 1342, 1425, 1468, 1515, 1630, 1712.

4.4.18. *N*3-(4-Methoxyphenyl)-6-methyl-2,5-dioxo-1,2,5,6-tetrahydrobenzo[*h*][1,6]naphthyridine-3-carboxamide (15hH). The title compound was obtained as pale yellow solid by microwave irradiation of 2 mmol (920 mg) of salt **19hH** in *i*-PrOH (2 mL) at 150 °C for 10 min. The same product was isolated when after the general procedure **4.4**. the reaction mixture was irradiated at 150 °C for 10 min in 48% yield, mp 340 °C dec. (recrystallized analytical sample). Anal. Calcd ($C_{21}H_{17}N_3O_4$): C, 67.19; H, 4.56; N, 11.19. Found: C, 67.11; H, 4.33; N, 11.41. ^{1}H NMR (DMSO-d_6) δ 3.71 (s, 3H), 3.75 (s, 3H), 6.95 (d, J=9.0 Hz, 2H), 7.48 (t, J=7.3 Hz, 1H), 7.65 (d, J=9.1 Hz, 2H), 7.71 (d, J=8.9 Hz, 1H), 7.88 (t, J=7.3 Hz, 1H), 8.16 (d, J=7.8 Hz, 1H), 8.83 (s, 1H), 10.35 (s, 1H); MS (pos. APCI) m/z 376 (M+1); IR (KBr), cm^{-1}: ν 1023, 1175, 1259, 1296, 1325, 1422, 1479, 1494, 1608, 1650, 2878, 2985, 3010.

4.4.19. *N*1-(4-Methoxyphenyl)-2-cyano-3-dimethylamino-2-propenamide (16H). A mixture of 1.0 mmol (190 mg) of *N*-(cyanoacetyl)-4-methoxyaniline **14H**, 1.0 equiv. (119 mg) of DMFDMA, 0.5 mL of *i*-PrOH and piperidine (2 drops ca. 28 mg) was irradiated under microwave conditions at 100 °C for 5 min in a 2.5 mL (small) Emrys™ process vial. After cooling, a white crystalline precipitate was formed, that was filtered off, washed with Et_2O and dried to give 160 mg (65%) yield of **16H** as a white solid. The same product was isolated when the general procedure **4.4**. was applied to acetophenone **11a** and nitrile **14H**. The formation of the intermediate adduct **13a** was confirmed by HPLC (Table 1) and mass spectrometry (pos. APCI) m/z 176 (M+1). Acetophenone was detected in the second reaction mixture by HPLC analysis. Product **16H**: ^{1}H NMR (DMSO-d_6) δ 3.20 (s, 3H), 3.26 (s, 3H), 3.71 (s, 3H), 6.84 (d, J=9.0 Hz, 2H), 7.45 (d, J=9.0 Hz, 2H), 7.79 (s, 1H), 8.91(s, 1H). MS (pos. APCI) m/z 246 (M+1).

4.4.20. *N*3-(4-Methoxyhenyl)-2-iminocoumarin-3-carboxamide (18H). An equimolar mixture of salicylaldehyde **17**, methylene active nitrile **14H** (2.0 mmol), *i*-PrOH (2 mL) and piperidine (2 drops, ca. 28 mg) was irradiated under microwave conditions at 100 °C for 1 min in a 5.0 mL Emrys™ Synthesizer process vial. Workup as described in the general procedure **4.4**. gave pure 2-iminocoumarine **18H**, mp 208 °C.[46] Anal. Calcd ($C_{17}H_{14}N_2O_3$): C, 69.38; H, 4.79; N, 9.52. Found: C, 69.43; H, 4.77; 9.46; ^{1}H NMR ($CDCl_3$) δ 3.82 (s, 3H), 6.91 (d, J=8.9 Hz, 2H) 7.16 (d, J=8.2 Hz, 1H), 7.23 (t, J=7.5 Hz, 1H), 7.41–7.59 (m, 2H), 7.63–7.82 (m, 3H), 8.57 (s, 1H), 12.42 (s, 1H); MS (pos. APCI) m/z 295 (M+1); IR (KBr), cm^{-1}: ν 1509, 1559, 1569, 1670, 3276.

4.4.21. Dimethylammonium 2-(2,2-dicyanovinyl)-5,5-dimethyl-3-oxo-1-cyclohexen-1-olate (19dA). To enamine **13d** (4.0 mmol) 2 mL of *i*-PrOH was added and the stirred mixture was cooled to 0 °C. Malonodinitrile (1.0 equiv., 264 mg) was added to the mixture, and within 2 min a white precipitate was formed that was filtered off after 2 h, washed with *i*-PrOH (1 mL), Et_2O and dried to give 670 mg (64%) of a white solid, mp 134 °C. Anal. Calcd ($C_{14}H_{19}N_3O_2$): C, 64.35; H, 7.33; N, 16.08. Found: C, 64.49; H, 7.48; N, 15.99; ^{1}H NMR (DMSO-d_6) δ 0.92 (s, 6H), 2.15 (s, 4H), 2.55 (s, 6H), 7.33 (s, 1H), 7.50 (br s,2H); ^{13}C NMR (DMSO-d_6) δ 28.7 (2Me), 30.7, 34.9 (NMe_2), 51.8 ($2CH_2$), 59.6, 113.0, 117.4, 122.2, 148.8 (CH), 193.7 (DEPT 135); MS (pos. APCI) m/z 217 (M+1-$HNMe_2$), MS (pos. ESI) m/z 262 (M+1); IR (KBr), cm^{-1}: ν 1403, 1502 1537, 1593, 2199, 2208, 2869, 2950.

4.4.22. Dimethylammonium 2-(2-carbamoyl-2-cyano-1-ethenyl)-5,5-dimethyl-3-oxo-1-cyclohexen-1-olate (19dC). The title compound was obtained from dimedone **11d** and cyanoacetomide **14C**, following general procedure **4.4**., using 2.0 mmol of starting materials, with an additional purification step: the crude isolated product in 2.0 mL of *i*-PrOH was irradiated under microwave conditions at 100 °C for 2 min. After cooling, the white precipitate was filtered off, washed with Et_2O and dried to give 312 mg (56%) of pure (^{1}H NMR) product. The same product was obtained when the equimolar mixture (4.0 mmol, each) of enamine **13d** and cyanoacetamide **14C**, containing piperidine (2 drops, ca. 28 mg) was stirred overnight at room temperature. The pure product (868 mg, 78%) was worked up as described in procedure **4.4**, mp 138 °C. Anal. Calcd ($C_{14}H_{21}N_3O_3$): C, 60.20; H, 7.58; N, 15.04. Found: C, 60.43; H, 7.28; N, 15.22; ^{1}H NMR (DMSO-d_6) δ 0.93 (s, 6H), 2.10 (s, 4H), 2.55 (s, 6H), 6.55 (br, s, 2H), 8.05 (s, 1H); MS (pos. API-ES) m/z 280 (M+1). IR (KBr), cm^{-1}: ν 1355, 1505, 1533, 1667, 2189, 2785, 2949, 3050, 3234, 3411.

4.4.23. Dimethylammonium 2-[2-cyano-2-(pyridin-2-ylcarbamoyl)-ethenyl]-5,5-dimethyl-3-oxo-cyclohexen-1-olate (19dI). Mp 136 °C. Anal. Calcd ($C_{19}H_{24}N_4O_3$): C, 64.03; H, 6.79; N, 15.72. Found: C, 64.13; H, 6.45; N, 15.94; ^{1}H NMR (DMSO-d_6) δ 0.93 (s, 6H), 2.10 (s, 4H), 2.55 (s, 6H), 6.55 (br, s, 2H), 8.05 (s, 1H); MS (pos. API-ES) m/z 357 (M+1). IR (KBr), cm^{-1}: ν 1287, 1309, 1408, 1583, 1667, 2197, 2954, 3025, 3246, 3361.

4.4.24. Open-chain piperidine salts (19fH, gH, hH). These salts were obtained as pale yellow solids from building blocks **11f–h**, DMFDMA **12** and the nitrile **14H**, following the general procedure **4.4**., using 2.0 mmol of starting materials and 1.0 equiv. of piperidine. Yields: **19fH** 68%, **19gH** 89%, **19hH** 75%.

4.4.25. Piperidinium 5-[2-cyano-2-(4-methoxyphenyl-carbamoyl)-1-ethenyl]-1,3-dimethyl-2,4-barbiturate (19fH). Mp 134 °C. Anal. Calcd ($C_{22}H_{27}N_5O_5$): C, 59.85; H, 6.16; N, 15.86. Found: C, 60.13; H, 6.21; N, 15.65; ^{1}H NMR (DMSO-d_6) δ 1.58 (m, 6H), 2.48 (s, 6H), 2.94 (m, 4H), 3.72 (s, 3H), 4.75 (br s, 2H), 6.86 (d, J=9.0 Hz, 2H), 7.53 (d, J=9.0 Hz, 2H), 8.25 (s, 1H), 8.92 (s, 1H); MS (pos. API-ES) m/z 442 (M+1). IR (KBr), cm−1: ν 1508, 1550, 1599, 1640, 1660, 2175, 2340, 2363, 2879, 2984, 3020.

4.4.26. Piperidinium 3-[2-cyano-2-(4-methoxy-phenyl-carbamoyl)-ethenyl]-2-oxo-2*H*-chromen-4-olate (19gH). Evaporation of solvent from the reaction mixture gave a viscous oil. HPLC/MS (pos. API-ES) m/z 448 (M+1) and the m/z for starting enamine **13g** and nitrile **14H** were not detected.

4.4.27. Piperidinium 3-[2-cyano-2-(4-methoxyphenyl-carbamoyl)-1-ethenyl]-1-methyl-2-oxo-1,2-dihydro-4-quinolinolate (19hH). Mp 138 °C. Anal. Calcd ($C_{26}H_{28}N_4O_4$): C, 67.81; H, 6.13; N, 12.17. Found: C, 67.63; H, 5.90; N, 11.94; ^{1}H NMR (DMSO-d_6) δ 1.58 (m, 6H), 2.49 (s, 2H), 2.99(m, 4H), 3.44 (s, 3H), 3.72 (s, 3H), 6.87 (d, J=8.7 Hz, 2H), 7.05 (t, J=7.3 Hz, 1H), 7.23 (d, J=8.3 Hz, 1H), 7.47 (t, J=7.2 Hz, 1H), 7.56 (d, J=8.7 Hz, 2H), 8.00 (d, J=7.6 Hz, 1H), 8.47 (s, 1H), 9.03 (s, 1H); MS (pos. APCI) m/z 461 (M+1); IR (KBr), cm^{-1}: ν 1037, 1178, 1225, 1304, 1409, 1508, 1555, 1592, 1667, 2190, 2345, 2366, 2879, 2984, 3019.

4.4.28. 5-(4-Methoxyanilinomethylene)-1,3-dimethyl-barbituric acid (20fH), 3-[(4-methoxyphenyl)amino]-methylene-2*H*-chromene-2,4(3*H*)-dione (20gH) and 3-[1-(4-methoxyanilino)methylidene]-1-methyl-1,2,3,4-tetrahydro-2,4-quinolinedione (20fH). The title compounds were obtained as pale yellow solids from building blocks **11f–h** and **14H**, following general procedure **4.4**., using 2.0 mmol of starting materials, and microwave conditions in the second step at 150 °C for 5 min. Product **20fH**: mp 161 °C. Anal. Calcd ($C_{14}H_{15}N_3O_4$): C, 58.13; H, 5.23; N, 14.53. Found: C, 58.11; H, 5.23; N, 14.52; ^{1}H NMR (DMSO-d_6) δ 3.16 (s, 3H), 3.17 (s, 3H), 3.75 (s, 3H), 6.97 (d, J=8.9 Hz, 2H), 7.45 (d, J=8.9 Hz, 2H), 8.45 (s, 1H), 11.88 (br s, 1H); MS (pos. APCI) m/z 290 (M+1); IR (KBr), cm^{-1}: ν 797, 834, 1028, 1176, 1205, 1314, 1341, 1426, 1475, 1634, 1657, 2950, 3012, 3410. Product **20gH**: mp 156 °C. Anal. Calcd ($C_{17}H_{13}NO_4$): C, 69.15; H, 4.44; N, 4.74. Found: C, 69.01; H, 4.23; N, 4.50; ^{1}H NMR (DMSO-d_6) δ 3.79 (s, 3H), 7.03 (d, J=8.9 Hz, 2H), 7.35 (m, 2H), 7.62 (d, J=8.9 Hz, 2H), 7.70(t, J=7.7 Hz, 1H), 7.97 (d, J=7.2 Hz, 2H), 8.79 (s, 1H), 11.90 (br, 1H); MS (pos. APCI) m/z 290 (M+1); IR (KBr), cm^{-1}: ν 1195, 1250, 1308, 1442, 1505, 1602, 1637, 1667, 2965, 3005, 3035, 3328. Product **20hH**, mp 154 °C; HPLC/MS (pos. API-ES) m/z 309 (M+1), the m/z for starting enamine **13g** and nitrile **14H** were not detected.

4.4.29. One-pot procedure for the synthesis of 2-pyrans (21fH, gH, hH). The intermediate salts were obtained as pale yellow solids from building blocks **11f–h** and **14H**, following general procedure **4.7**. To the final reaction mixture conc. HCl (1 mL) was added and it was irradiated again with microwaves at 120 °C for 2 min and worked up as described in procedure **4.4**.

4.4.30. *N*6-(4-Methoxyphenyl)-1,3-dimethyl-2,4,7-trioxo-1,3,4,7-tetrahydro-2*H*-pyrano-[2,3-*d*]pyrimidine-6-carboxamide (21fH). Mp 262 °C. Anal. Calcd ($C_{17}H_{15}N_3O_6$): C, 57.17; H, 4.23; N, 11.76. Found: C, 56.89; H, 4.33; N, 12.05; ^{1}H NMR (DMSO-d_6) δ 3.12 (s, 6H), 3.71 (s, 3H), 6.85 (d, J=9.0 Hz, 2H), 7.52 (d, J=9.0 Hz, 2H), 8.24 (s, 1H), 8.90 (s, 1H); MS (neg. APCI) m/z 356.

4.4.31. *N*3-(4-Methoxyphenyl)-2,5-dioxo-2*H*,5*H*-pyrano[3,2-*c*]chromene-3-carboxamide (21gH). Mp 259 °C. Anal. Calcd ($C_{20}H_{13}NO_6$): C, 66.12; H, 3.61; N, 3.86. Found: C, 66.32; H, 3.57; N, 4.01; ^{1}H NMR (DMSO-d_6) δ 3.75 (s, 3H), 6.95 (d, J=9.0 Hz, 2H), 7.58 (m, 2H), 7.64 (d, J=9.0 Hz, 2H), 7.88 (t, J=6.5 Hz, 1H), 8.10 (d, J=6.7 Hz, 1H), 8.61 (s, 1H), 10.26 (s, 1H); MS (pos. APCI) m/z 364 (M+1).

4.4.32. *N*3-(4-Methoxyphenyl)-6-methyl-2,5-dioxo-5,6-dihydro-2*H*-pyrano[3,2-*c*]quinoli-ne-3-carboxamide (21hH). Mp 256 °C dec. Anal. Calcd ($C_{21}H_{16}N_2O_5$): C, 67.02; H, 4.28; N, 7.44. Found: C, 66.72; H, 4.24; N, 7.55. ^{1}H NMR (DMSO-d_6) δ 3.61 (s, 3H), 3.74 (s, 3H), 6.94 (d, J=8.9 Hz, 2H), 7.45 (t, J=7.1 Hz, 1H), 7.63 (d, J=8.9 Hz, 2H), 7.69 (d, J=8.7 Hz, 1H), 7.85 (t, J=7.1 Hz, 1H), 8.15 (d, J=7.1 Hz, 1H), 8.88 (s, 1H), 10.33 (s, 1H); MS (pos. APCI) m/z 377 (M+1).

4.4.33. Dimethylammonium 3-cyano-7,7-dimethyl-5-oxo-1,2,5,6,7,8-hexahydro-2-quinolin-4-olate (22dA). The title compound was obtained as white solid from **13d** and malonodinitrile **14A**, following general procedure **4.4**., using 4.0 mmol of starting materials; yield 575 mg (55%). The same compound was obtained by irradiation of 430 mg (1.76 mmol) of salt **19dA** in *i*-PrOH (2 mL) with microwave irradition at 130 °C for 10 min, yield 260 mg (50%), mp 214 °C. Anal. Calcd ($C_{14}H_{19}N_3O_2$): C, 64.35; H, 7.33; N, 16.08. Found: C, 64.17; H, 7.28; N, 16.36; ^{1}H NMR ($CDCl_3$) δ 1.11 (s, 6H), 2.44 (s, 2H), 2.74 (s, 2H), 2.80 (s, 6H), 6.36 (br s, 2H), 8.34 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 28.4 (2Me), 32.6, 34.7 (NMe_2), 45.6 (CH_2), 51.2 (CH_2), 96.7, 113.0, 119.2, 142.1, 167.2, 170.1, 193.5 (DEPT 135); MS (pos. APCI) m/z 217 (M+1-HNMe$_2$), MS (pos. ESI) m/z 217, 234, 239, 262 (M+1), 433, 455; IR (KBr), cm^{-1}: ν 1415, 1488, 1579, 1667, 2206, 2789, 2385, 2949, 3051.

4.4.34. 2,2-Dimethyl-4-oxo-1,2,3,4-tetrahydro-benzo[4,5]imidazo[1,2-*a*]quinolin-6-yl cyanide (23dJ). The title compound was obtained as pale green–yellow solid from dimedone **11d**, DMFDMA **12** and 2-cyano-methylbenzoimidazole (**14J**), following general procedure **4.4**., using 2.0 mmol of starting materials. Yield 450 mg (78%); mp 330–333 dec. Anal. Calcd ($C_{18}H_{15}N_3O$): C, 74.72; H, 5.23; N 14.52. Found: C, 74.66; H, 5.22; N 14.50; ^{1}H NMR (DMSO-d_6) δ: 1.19 (s, 6H), 2.62 (s, 2H), 3.73 (s, 2H), 7.54 (t, J=7.8 Hz, 1H), 7.69 (t, J=7.7 Hz, 1H), 8.00 (d, J=8.1 Hz, 1H), 8.46 (d, J=8.5 Hz, 1H), 8.51 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 28.5 (2Me), 33 (C-2), 41 (CH_2-1), 50 (CH_2-3), 99 (C-6), 115 (CN), 116 (C-4a), 117 (CH-11), 120 (CH-8), 123 (CH-10), 127 (CH-9), 131 (C-11a), 135 (CH-5), 145 (C-7a), 147 (C-6a), 154.5 (C-12a), 194

(CO-4); MS (pos. APCI) *m/z* 290 (M+1); IR (KBr), cm^{-1}: ν1515, 1558, 1592, 1688, 2279, 2962, 2865.

4.4.35. 1-(4-Methoxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydro-3-quinoline-carboxamide (24dC). In a small Emrys™ process vial containing salt **19dC** (140 mg, 0.5 mmol) and AcOH (0.5 mL) 4-methoxyaniline (37 mg, 1.5 equiv.) was added and the mixture was irradiated with microwave irradiation at 130 °C for 3 min. After cooling 0.5 mL of water was added to the stirred mixture, and the white compound was isolated as described in the general procedure **4.4**, mp 196 °C. Anal. Calcd ($C_{19}H_{20}N_2O_4$) C, 67.05; H, 5.92; N, 8.23. Found: C, 66.93; H, 5.99; N, 8.56; ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 6H), 2.42 (s, 2H), 2.45 (s, 2H), 3.91 (s, 3H), 5.73 (br s, 1H), 7.12 (s, 4H), 8.94 (br s, 1H), 9.20 (s, 1H); ^{13}C NMR (DMSO-d_6) ($CDCl_3$) δ 28.3, 29.7, 32.9, 42.9, 50.1, 55.6, 114.2, 115.7, 128.4, 129.3, 141.8, 158.8, 160.3, 164.6; MS (pos. APCI) *m/z* 341 (M+1).

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Synthesis of complex alkoxyamines using a polymer-supported *N*-hydroxyphthalimide

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Abstract—The synthesis of a polymer-supported *N*-hydroxyphthalimide is described. The polystyrene-bound *N*-hydroxyphthalimide resin **1** has been used to prepare complex alkoxyamines exhibiting both stereochemical and positional diversity. Methods for efficient condensation of complex alkoxyamines with aldehydes and ketones are also outlined.

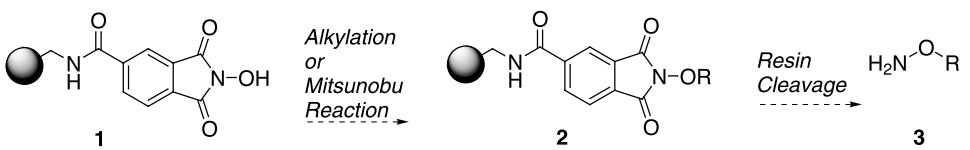
1. Introduction

The oxime functional group is present in several natural products and biologically active molecules.[1] The oxime ether is an attractive functionality to incorporate into library synthesis due to the chemoselective reaction of alkoxyamines[2] with aldehydes and ketones.[3] However, only a limited number of alkoxyamines are commercially available[4] and relatively few methods have been reported for the preparation of chiral variants.[5]

As part of our general interest to construct complex chemical libraries that contain both stereochemical[6] and positional diversification elements, we devised an approach towards the synthesis of stereochemically diverse alkoxyamines employing a polymer-supported *N*-hydroxyphthalimide (Scheme 1).[7,8] Alkylation of the phthalimide hydroxyl group using alkyl halides[9] or alcohols via Mitsunobu etherification[10] would provide the corresponding polymer-supported *N*-alkoxyphthalimides. These products could be further modified using solid-phase synthesis. Hydrazine-mediated cleavage of the alkylation products would afford the corresponding alkoxyamines. This paper reports the synthesis of a polystyrene-based *N*-hydroxyphthalimide resin and its utility in preparing stereochemically and structurally diverse alkoxyamines. The alkoxyamines were utilized to synthesize complex oxime ethers from aldehydes and ketones.

2. Results and discussion

A report from Aronov and Gelb[11] describing the preparation of a polystyrene-bound phthalimide reagent served as a starting point for preparation of *N*-hydroxyphthalimide resin **1**. The synthesis of **1** began by transformation of commercially available anhydride **4** to the *N*-hydroxyphthalimide **5** using hydroxylammonium chloride in refluxing pyridine[12] (Scheme 2). Protection of the hydroxyl group with trityl chloride afforded protected phthalimide **6**. Attachment of the protected phthalimide **6** to aminomethyl polystyrene (PS-NH_2, 1.30 mmol/g, Argonaut) using HATU-mediated amide bond formation afforded resin **7**. Deprotection of the trityl group using 10:1 CH_2Cl_2:TFA completed the synthesis of *N*-hydroxyphthalimide resin **1**. Unfortunately, extensive screening of reaction conditions



Scheme 1. Preparation of complex alkoxyamines using a polymer-supported *N*-hydroxyphthalimide.

Keywords: Polymer-supported; *N*-Hydroxyphthalimide; Alkoxyamine; Stereochemical diversity; Positional diversity.
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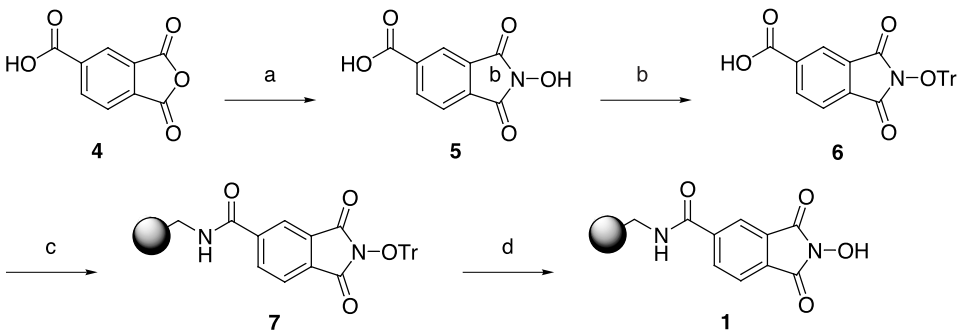


Scheme 2. Initial synthesis of an *N*-hydroxyphthalimide resin. Reagents and conditions: (a) $H_2NOH{\cdot}HCl$, pyridine, 95 °C, 66%. (b) TrCl, DIEA, CH_3CN, reflux, 34%. (c) HATU, DIEA, PS-NH_2, DMF. (d) 10:1 CH_2Cl_2:TFA.

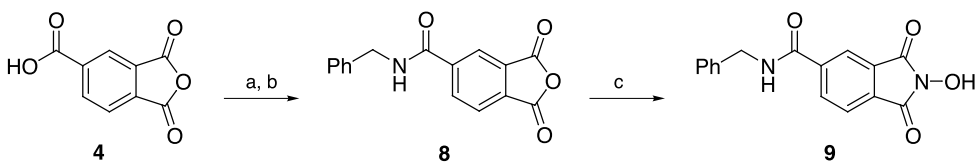
failed to improve the yield for trityl protection of *N*-hydroxyphthalimide **5**. Other protecting groups (e.g., TMS, TBS, and THP) were also evaluated and found to be less effective than the trityl group. Attempts to directly condense anhydride **4** with *O*-trityl hydroxylamine or *O*-(tetrahydro-2*H*-pyran-2-yl) hydroxylamine were likewise unsuccessful.

To improve the synthesis of resin **1**, amide bond formation in the presence of the anhydride moiety, followed by transformation to the hydroxyphthalimide, was investigated (Scheme 3). A model study was initiated to determine the effectiveness of amide bond formation with the acid chloride of compound **4**.[13] In order to potentially minimize reaction with the anhydride moiety, we employed benzylamine hydrochloride as a protected form of the amine. Slow addition of triethylamine gradually liberated the benzylamine to selectively react with the more reactive acid chloride. According to ^1H NMR analysis, no reaction of the anhydride was observed using 1 equiv. of the amine salt. Although this transformation provided only moderate yields, it was anticipated that by using an excess of acid chloride, high levels of chemoselectivity should be achievable using a solid-supported amine. Following a literature procedure,[12] anhydride **8** was reacted with hydroxylamine hydrochloride to provide the desired model compound **9**.

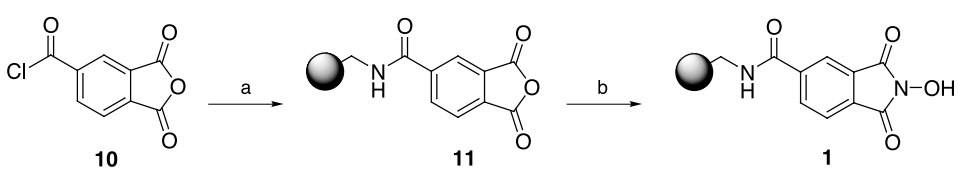
Following solution phase model studies, solid-phase synthesis was initiated by condensation of acid chloride **10** and PS-$NH_2{\cdot}HCl$, prepared from commercially available aminomethylated polystyrene (1.10 mmol/g, Novabiochem) and 4 M HCl in dioxane. The reaction was monitored by both bead staining with bromophenol blue[14] and single-bead IR analysis. A negative result from the bromophenol blue stain test and strong carbonyl absorptions (1858, 1781, 1673 cm^{-1}) by IR analysis clearly indicated the formation of anhydride resin **11**.[15] After resin washing, **11** was successfully transformed to *N*-hydroxyphthalimide resin **1** as indicated by the shift of the carbonyl stretches (1788, 1728, 1603 cm^{-1}) and the appearance of a strong hydroxyl stretch by single-bead IR (Scheme 4).

In order to determine the loading of *N*-hydroxyphthalimide resin **1**, a Mitsunobu reaction was employed for resin derivatization (Scheme 5). 4-Biphenylmethanol was reacted with resin **1** using TMAD/PBu_3.[16] After resin washing and subsequent cleavage using NH_2NH_2 (2 equiv.), the corresponding biaryl alkoxyamine[17] was obtained after solvent removal. The yield was determined by HPLC analysis of the acetone oxime derivative **13** employing 4-biphenylmethanol as an internal standard. The loading of resin **1** was thus determined to be 0.68 mmol/g (75% of theoretical loading). Further efforts to improve the loading of the resin were investigated. To minimize potential crosslinking of the anhydride with aminomethyl sites on the resin, other variables including use of a secondary amine resin[15] and alternative solvents (e.g., THF) were evaluated. However, none of these variations afforded improved resin loading.

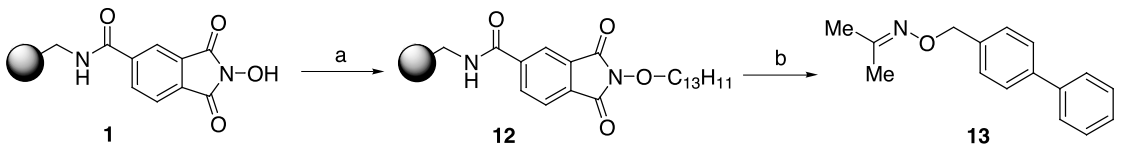
A recent report[18] prompted us to investigate an alternative



Scheme 3. Synthesis of *N*-hydroxyphthalimide resin via a direct condensation pathway. Reagents and conditions: (a) oxalyl chloride, CH_2Cl_2, cat. DMF. (b) $PhCH_2NH_2{\cdot}HCl$, Et_3N, CH_2Cl_2. (c) $H_2NOH{\cdot}HCl$, pyridine, 95 °C, 45% from **4**.



Scheme 4. Second synthesis of *N*-hydroxyphthalimide resin. Reagents and conditions: (a) PS-NH_2, Et_3N, CH_2Cl_2. (b) $H_2NOH{\cdot}HCl$, pyridine:$ClCH_2CH_2Cl$ 3:1, 75 °C.



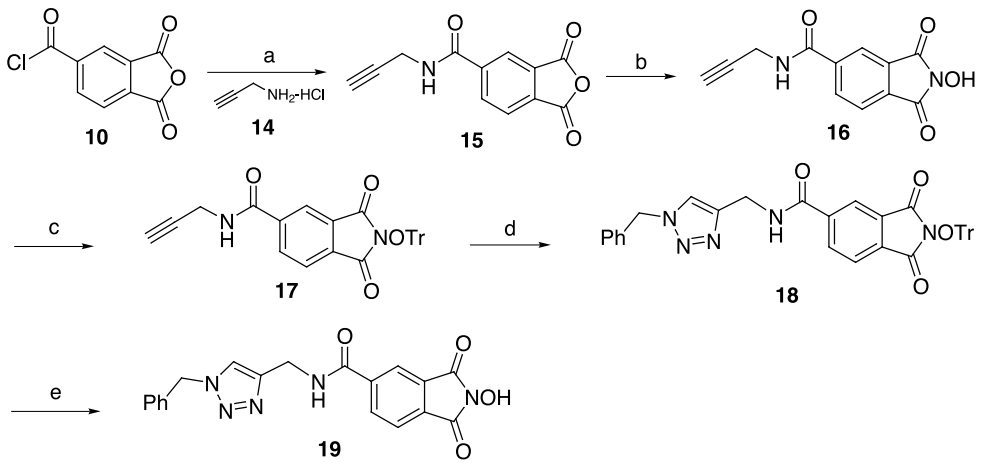
Scheme 5. Characterization of the *N*-hydroxyphthalimide resin. Reagents and conditions: (a) 4-biphenylmethanol, PBu_3, TMAD, THF:CH_2Cl_2=1:1, rt. (b) H_2NNH_2, CH_2Cl_2, then acetone.

polymer-supported reagent synthesis involving 1,2,3-triazole formation (Scheme 6). Selective condensation of acid chloride **10** and propargyl amine hydrochloride **14** afforded anhydride **15**, which was converted to hydroxyphthalimide **16** using microwave conditions (150 W, 100 °C, 60 min).[19] At this stage, both CuI-catalyzed[23a] and thermal triazole formations were evaluated and failed to provide any desired product. Further studies indicated that the trityl-protected compound **17**[20] (prepared from **16** and trityl chloride) readily underwent cycloaddition using Cu(I) catalysis. Interestingly, a mixture of 1.4:1 mixture of 1,2,3-triazole regioisomers (unassigned) was obtained instead of the expected head-to-tail regioisomer.[23] Subsequent deprotection of both isomers using 10:1 CH_2Cl_2/TFA successfully afforded the desired model 1,2,3-triazole **19**.

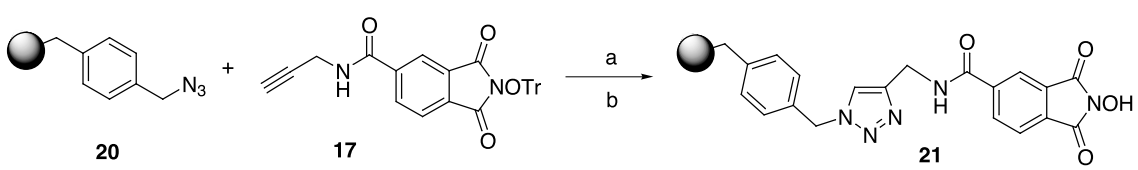
Following the solution phase model studies, compound **17** was employed for solid-phase synthesis (Scheme 7). Copper (I)-mediated dipolar cycloaddition of benzyl azide resin **20**[18,21] and propargylamide **17**, followed by TFA-mediated detritylation, afforded the desired resin **21**.[22] Although Mitsunobu reactions proceeded smoothly using **21**, subsequent functionalization reactions to produce complex alkoxyamines (acetylenic Mannich reaction, vide infra) produced inconsistent results leading to general concern about 1,2,3-triazole regioisomers and potential for compromised reactivity. Accordingly, we selected *N*-hydroxyphthalimide resin **1** for further studies to prepare structurally and stereochemically diverse alkoxyamines.

Due to the high efficiency of the Mitsunobu reaction and the numerous transformations that potentially can be applied on tethered alkynes, we focused on attachment of alkyne-containing alcohols to resin **1**. Three diversification pathways were selected for terminal alkyne modification: Cu-catalyzed 1,2,3-triazole formation,[23] isoxazole synthesis,[24] and acetylenic Mannich reactions[25] (Scheme 8).

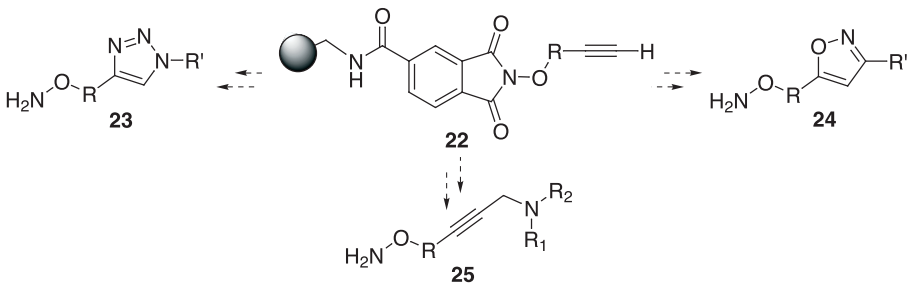
Condensation of *N*-hydroxyphthalimide resin **1** and diverse terminal alkyne-containing alcohols was effected using a Mitsunobu conditions[16] to afford alkyne resins **26** (Scheme 9). At this stage, Cu-catalyzed 1,2,3-triazole formation was investigated.[23] A number of Cu(I) sources were tested along with variation of base, additives, and ligands. The cycloaddition was monitored by the disappearance of alkyne C–H stretch (3292 cm^{-1}) using single-bead IR. Optimal reaction conditions were found using a combination of $Cu(MeCN)_4PF_6$, 2,6-lutidine, and CH_2Cl_2 as solvent. Resin washing with 10% *N*,*N*,*N*′,*N*′-tetramethylethylenediamine efficiently removed the copper



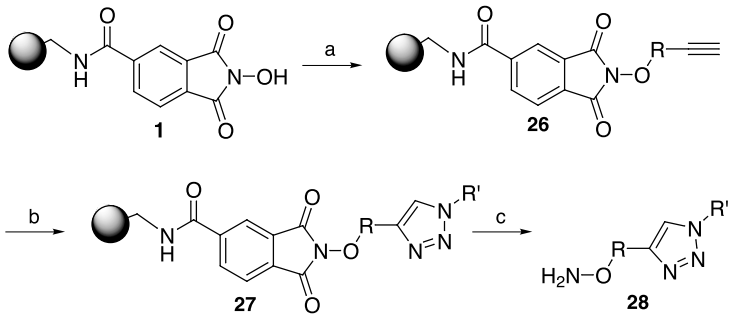
Scheme 6. Model studies towards the synthesis of an *N*-hydroxyphthalimide resin using 1,2,3-triazole linkage (only one regioisomer of **18** and **19** are shown for clarity). Reagents and conditions: (a) pyridine, THF. (b) $NH_2OH{\cdot}HCl$, pyridine, microwave 100 °C, 150 W, 60 min 75%. (c) TrCl, DIEA, MeCN. (d) CuI, DIEA, THF, $PhCH_2N_3$. (e) 10:1 CH_2Cl_2:TFA, 90% from **16**.



Scheme 7. Synthesis of an *N*-hydroxyphthalimide resin using a triazole linker (only one regioisomer **21** is shown for clarity). Reagents and conditions: (a) CuI, DIEA, THF. (b) 10:1 CH_2Cl_2:TFA.



Scheme 8. Plan for synthesis of complex alkoxyamines using the *N*-hydroxyphthalimide resin.



Scheme 9. Synthesis of 1,2,3-triazole-containing alkoxyamines. Reagents and conditions: (a) alkyne-containing alcohol, TMAD, Bu_3P, THF/CH_2Cl_2=1:1, rt. (b) R'N_3, Cu(MeCN)$_4PF_6$, 2,6 lutidine, CH_2Cl_2. (c) NH_2NH_2, CH_2Cl_2.

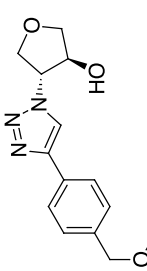
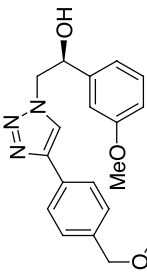
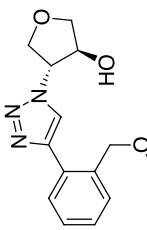
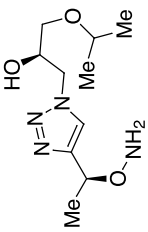
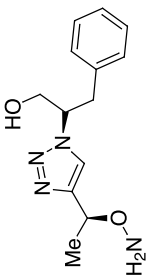
salts from the polystyrene support. Subsequent cleavage using anhydrous hydrazine provided the desired products. Using this protocol, several alkoxyamine triazoles (Table 1, **29**–**33**) containing both positional and stereochemical diversity were prepared in yields ranging from 95 to 98% (71–74% based on loading of the aminomethylated polystyrene) and high purities (>99%, HPLC-ELSD).

Isoxazoles are heterocycles which are present in a number of therapeutic agents.[26] Thus, the cycloaddition of nitrile oxides with alkynes was investigated for preparation of diverse isoxazole-containing alkoxyamines (Scheme 10).[24] Dipolar cycloaddition of alkyne resin **26** was best performed using in situ generation of a nitrile oxide prepared from oxime **35**, NBS, and $NaHCO_3$[27] in CH_2Cl_2 at 35 °C. Other conditions[24c,28] employing NCS,[24a] NaOCl,[24b] and $(Bu_3Sn)_2O$[29] lead to incomplete reactions as evidenced by single bead IR. Regioisomers were observed for some of the substrates (Table 2) and the ratio was determined by ^1H NMR. Due to the limited availability of diverse oximes, we have developed a practical method for the synthesis of oximes starting from commercially available aldehydes (inset, Scheme 10). Treatment of aldehydes **34** with hydroxylamine hydrochloride in the presence of Et_3N in CH_2Cl_2 provided the desired oximes **35**, which can be readily separated from the hydrochloride salts by eluting though an SLE[30] cartridge containing 1 N HCl. The resulting oximes were reacted with NBS in CH_2Cl_2 for 3 h and then incubated with diverse alkyne resins ($NaHCO_3$, 12 h) to afford the corresponding isoxazole-containing resins. After hydrazinolysis, the desired isoxazole-containing alkoxyamines (Table 2, **38**–**42**) were obtained in yields ranging from 92 to 96% (69–72% based on loading of the aminomethylated polystyrene) and high purities (≥92%, HPLC-ELSD).

Since tertiary propargylic amines are also of significant pharmaceutical interest,[31] the preparation of propargylic amine-containing alkoxyamines was next explored (Scheme 11). Initial reactions employing conventional reaction conditions (e.g., CuCl/1,4-dioxane[25]) were not satisfactory and led to incomplete reactions. After extensive experimentation, we found that pretreating the secondary amine with paraformaldehyde and *p*-toluenesulfonic acid, followed by addition of CuBr in DMF, provided the desired Mannich products in high yield and purity after cleavage with NH_2NH_2. The washing regimen developed for the solid phase 1,2,3-triazole synthesis (Scheme 9) was also employed for removal of copper salts from the polystyrene support. Following this general procedure, several propargylic amine-containing alkoxyamines (Table 3, **46**–**50**) were synthesized employing commercially available amines in yields ranging from 95 to 99% (71–74% based on loading of the aminomethylated polystyrene) and high purities (≥97%, HPLC-ELSD).

The condensation of alkoxyamines with aldehydes or ketones via oxime formation is an efficient method for the ligation of two complex fragments (Scheme 12). A small collection of oxime–ether containing molecules was targeted to demonstrate the utility of the complex alkoxyamines for preparation of compounds with stereochemical and positional diversity. Microwave irradiation[32] was employed to facilitate expedient product formation. General conditions for the preparation of oxime ethers employed a slight excess of alkoxyamine (1.2 equiv.) relative to ketone or aldehyde. Aldehydes (**51**–**53**) reacted cleanly with alkoxyamines in 1,2-dichloroethane to produce the desired oxime products (**57**–**59**) within minutes (Table 4). Excess alkoxyamine was scavenged using a solid-supported methyl isatoic anhydride resin[33] to afford the

Table 1. Synthesis of 1,2,3-triazole-containing alkoxyamines

Substrate					
	29	**30**	**31**	**32**	**33**
Yield (%)[a]	97	98	96	95	98
Purity (%)[b]	>99	>99	>99	>99	>99

[a] Isolated yield based on the loading of the *N*-hydroxyphthalimide resin **1** (0.68 mmol/g).
[b] Purity determined by HPLC-ELSD analysis of the corresponding acetone oxime.

desired products in high yield and purity. The stereochemistry of oxime ethers was determined by ^{1}H NMR analysis.[1a] Higher reaction temperatures and use of acetic acid improved the rate of oxime condensations with ketones such as estrone **54** and (+)-griseofulvin[34] **55** to afford compounds **60** and **61**. Production of structures such as **57**–**61** using oxime ligation illustrates the potential for future synthesis of complex hybrid molecule libraries using convergent approaches.[35]

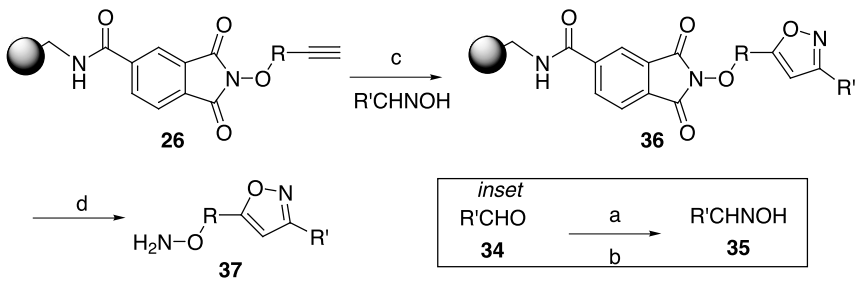
3. Conclusion

In summary, the synthesis of a polymer-supported *N*-hydroxyphthalimide has been accomplished. In initial studies, the supported *N*-hydroxyphthalimide reagent has been used to prepare complex alkoxyamines containing stereochemical and positional diversity elements. Attachment of alkynols to the *N*-hydroxyphthalimide resin using Mitsunobu reactions has facilitated the synthesis of complex 1,2,3-triazole-, isoxazole- and propargylamine-containing alkoxyamines via branching reaction pathways. Finally, methods for efficient condensation of the diverse alkoxyamines with both aldehydes and ketones have been developed leading to the production of highly complex, hybrid molecules. Further studies concerning the construction of complex oxime-based libraries using convergent approaches is currently in progress and will be reported in due course.

4. Experimental

4.1. General experimental procedures

^{1}H NMR spectra were recorded on a 400 MHz spectrometer at ambient temperature with $CDCl_3$ as the solvent unless otherwise stated. ^{13}C NMR spectra were recorded on a 75.0 MHz spectrometer (unless otherwise stated) at ambient temperature. Chemical shifts are reported in parts per million relative to chloroform (^{1}H, δ 7.24; ^{13}C, δ 77.23). Data for ^{1}H NMR are reported as follows: chemical shift, integration, multiplicity (app=apparent, par obsc=partially obscure, ovrlp=overlapping, s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet) and coupling constants. All ^{13}C NMR spectra were recorded with complete proton decoupling. Infrared spectra were recorded on a Nicolet Nexus 670 FT-IR spectrophotometer. Single-bead IR was performed on Nicolet Nexus 470 FT-IR+ContinuμM microscope. Optical rotations were recorded on an AUTOPOL III digital polarimeter at 589 nm, and are recorded as $[\alpha]_D$ (concentration in grams/100 mL solvent). HPLC-ELSD-MS analysis was performed on a Waters HPLC-MS system equipped with Waters 600 HPLC pump, Waters 2996 photodiode array detector, Micromass ZQ Quadrupole Mass Spectrometer, Sedere Sedex 75 ELS reverse phase column (XTerra®RP_8, 5 μm, 4.6×30 mm). Analytical thin layer chromatography was performed on 0.25 mm silica gel 60-F plates. Flash chromatography was performed using 200–400 mesh silica gel (Scientific Absorbents, Inc.). Solid-phase synthesis was performed using Quest 210 and Quest 205 synthesizer (Argonaut Technologies, Foster City, CA). Liquid–liquid extraction



Scheme 10. Synthesis of isoxazole-containing alkoxyamines. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, CH_2Cl_2, Et_3N. (b) SLE, 1 N HCl. (c) NBS, CH_2Cl_2, then $NaHCO_3$, 35 °C. (d) NH_2NH_2, CH_2Cl_2.

for oxime formation was performed using 5.0 mL ISOLUTE® HM-N SPE Columns (Argonaut Technologies). Aminomethylated polystyrene (cat. No. 01-64-0143, 1.10 mmol/g) was obtained from Novabiochem. Polystyrene-NH_2 resin (PS-NH_2, part. no. 800263, 1.30 mmol/g) was obtained from Argonaut Technologies. Solid-supported methyl isatoic anhydride resin (PL-MIA, part # 3405-3679, 2.57 mmol/g) was obtained from Polymer Laboratories. All other reagents were used as supplied by Sigma-Aldrich, Lancaster Synthesis, Strem, and Argonaut Technologies. Methylene chloride, tetrahydrofuran, methanol, benzene were purified by passing through two packed columns of neutral alumina (Glass Contour, Irvine, CA).

4.1.1. *N*-Hydroxyphthalimide resin (1). Pre-swelled aminomethylated polystyrene resin (5.0 g, 5.50 mmol, loading: 1.10 mmol/g,) was mixed with 40 mL CH_2Cl_2 in an 80 mL reaction vessel on the Quest 205 parallel synthesizer. After addition of 6.9 mL HCl in dioxane (4.0 M, 27.4 mmol), the mixture was agitated for 1 h. Upon filtration, the PS-$NH_2{\cdot}HCl$ resin was washed with CH_2Cl_2 (3×30 mL) and transferred to a pre-silylated 250 mL two-neck flask equipped with a mechanical stirrer. Trimellitic anhydride chloride (5.75 g, 27.4 mmol) was added followed by 30 mL CH_2Cl_2 and the reaction was cooled to 0 °C using an ice bath. A solution of Et_3N (5.26 mL, 38.5 mmol) in 20 mL CH_2Cl_2 was added to the reaction mixture over 2 h via syringe pump. The reaction was stirred at 0 °C for an additional 3 h, warmed to rt, and stirred for an additional 2 h. After the reaction mixture was transferred to a reaction vessel on the Quest 205 synthesizer, the resulting anhydride resin was washed with CH_2Cl_2 (2×20 mL), DMF (2×20 mL), THF (2×20 mL), and CH_2Cl_2 (2×20 mL). Following washing, $NH_2OH{\cdot}HCl$ (1.91 g, 27.5 mmol) and 40 mL 3:1 pyridine: 1,2-dichloroethane were added and the reaction was agitated for 24 h at 75 °C. The reaction was cooled to rt, the resin was filtered, washed with MeOH (2×20 mL), DMF (2×20 mL), DMF–H_2O=1:1 (2×30 mL), DMF (1×20 mL), THF (3×20 mL), CH_2Cl_2 (2×20 mL), Et_2O (2×20 mL), and dried under high vacuum at 50 °C for 12 h. IR (single bead): 3385, 3060, 3027, 2925, 1788, 1728, 1669, 1601, 1514, 1494, 1453, 1374, 1299, 1194, 1141, 1115, 1021, 998, 913, 827, 761, 709 cm^{-1}.

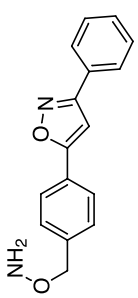
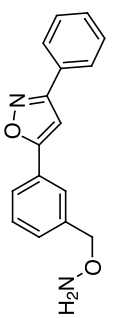
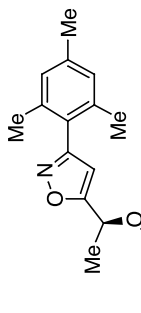
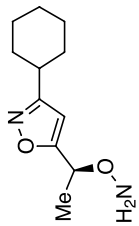
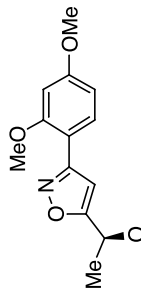
4.2. Loading determination

Resin **1** (100 mg) was pre-swelled with CH_2Cl_2 in a 5 mL reaction vessel on the Quest-210 synthesizer and 4-biphenylmethanol (55.2 mg, 0.30 mmol) and azodicarboxylic acid bis[dimethylamide] (51.6 mg, 0.30 mmol) were added as solids under N_2 followed by 2 mL 1:1 THF–CH_2Cl_2. After mixing to dissolve the reagents, P(*n*-Bu)$_3$ (43.2 μL, 0.30 mmol) was added and reaction mixture was agitated for 6 h. After filtering the reaction mixture, the resulting resin was washed with DMF (2×3 mL), MeOH (2×3 mL), THF (3×3 mL), CH_2Cl_2 (3×3 mL). Following the addition of 2 mL CH_2Cl_2, NH_2NH_2 (6.3 μL, 0.20 mmol) was added and the reaction was agitated for 1 h. The filtration was collected and treated with 2 mL acetone. After concentration, the resulting sample was subjected to an HPLC analysis using a Waters AutoPure HPLC systemand an XTerra®RP_8, 5 μm, 4.6×30 mm column (4-biphenylmethanol as internal standard). The loading was determined to be 75% (0.68 mmol/g).

Note. For compounds **29**–**33**, **38**–**42**, and **46**–**50** (a) all yields were calculated based on the experimentally determined loading of the *N*-hydroxyphthalimide resin (**1**, cf. Section 4.1.2); (b) purity and NMR studies were conducted on the corresponding acetone oxime derivatives.

4.2.1. Representative procedure for synthesis of 1,2,3-triazole-containing alkoxyamines (acetone oxime of 33). Resin **1** (56 mg, 0.0385 mmol) was pre-swelled with CH_2Cl_2 in a 5 mL reaction vessel on the Quest-210 synthesizer. Azodicarboxylic acid bis[dimethylamide] (20 mg, 0.116 mmol) was added as a solid under N_2 followed by 2 mL 1:1 THF–CH_2Cl_2 and (*R*)-(+)-3-butyne-2-ol (9.1 μL, 0.116 mmol). After mixing to dissolve the reagents, P(*n*-Bu)$_3$ (16.4 μL, 0.116 mmol) was added and reaction mixture was agitated for 6 h. After filtering the reaction mixture, the resulting resin was washed with DMF (2×3 mL), MeOH (2×3 mL), THF (3×3 mL), and CH_2Cl_2 (3×3 mL). After resin washing, $Cu(CH_3CN)_4PF_6$ (4.5 mg, 0.012 mmol, 30 mol%) was added to the reaction vessel followed by the addition of 2 mL CH_2Cl_2, 2*S*-2-azido-3-phenyl-propan-1-ol[36] (20.5 mg, 0.116 mmol) and 2,6-lutidine (13.5 μL, 0.116 mmol). The reaction was agitated at rt for 12 h and filtered. After resin washing with 10% *N*,*N*,*N*′,*N*′-tetramethylethylenediamine in CH_2Cl_2 (2×3 mL), CH_2Cl_2 (1×3 mL), 5% AcOH in CH_2Cl_2 (2×3 mL), THF (3×3 mL) and CH_2Cl_2 (3×3 mL), 2 mL CH_2Cl_2 and NH_2NH_2 (2.5 μL, 0.077 mmol) were added and the reaction was agitated for 1 h. The reaction was filtered, the resin was washed with CH_2Cl_2, and the filtrate collected and evaporated to afford 9.9 mg (0.0377 mmol, 73.5%) of (2*R*)-[4-[(1*S*)-aminooxy-ethyl)-[1,2,3]triazol-1-yl]-3-phenyl-propan-1-ol **33** as a colorless oil. **33** was treated with 2 mL of acetone and then concentrated and the

Table 2. Synthesis of isoxazole-containing alkoxyamines

Substrate					
	38	**39**[a]	**40**[b]	**41**	**42**
Yield (%)[c]	95	92	93	96	95
Purity (%)[d]	>99	>99	92	>99	>99

[a] 2:1 mixture of regioisomers (major isomer shown).
[b] >10:1 mixture of regioisomers (major isomer shown).
[c] Isolated yield based on the loading of the *N*-hydroxyphthalimide resin **1** resin (0.68 mmol/g).
[d] Purity determined by HPLC-ELSD analysis of the corresponding acetone oxime.

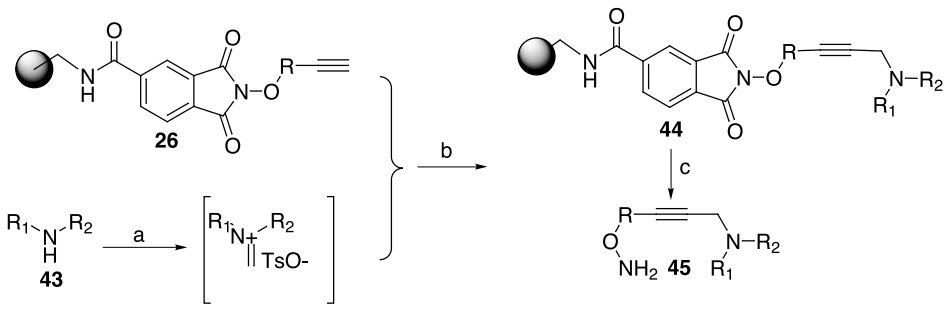
resulting sample was subjected to HPLC-ELSD analysis using a Waters HPLC-MS system (XTerra®RP_8, 5 μm, 4.6×30 mm column) to determine the purity (>99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28–7.20 (m, 3H), 7.08 (d, 2H, J=7.2 Hz), 5.32 (q, 1H, J=7.2 Hz), 4.67 (m, 1H), 4.07 (m, 2H), 3.25 (m, 2H), 2.01 (m, 1H), 1.84 (s, 3H), 1.83 (s, 3H), 1.61 (d, 3H, J=6.8 Hz); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 155.3, 149.4, 136.6, 129.1, 128.8, 127.0, 122.1, 73.1, 64.7, 63.6, 37.9, 21.8, 19.8, 15.7; IR (thin film) ν_{max} 3333, 3029, 2926, 2857, 1454, 1372, 1079, 935, 702, 670 cm^{-1}; LRMS $[M]^+$ calculated for $C_{16}H_{22}N_4O_2$: 302.4, found: 302.5; $[\alpha]_D^{23}=-57.8°$ (c=0.58, CH_2Cl_2).

4.2.2. (4*R*)-[4-(4-Aminooxymethyl-phenyl)-[1,2,3]triazol-1-yl]-tetrahydro-furan-(3*R*)-ol (acetone oxime of 29). White solid. Mp 125.0–126.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.73 (d, 2H, J=7.6 Hz), 7.39 (d, 2H, J=7.6 Hz), 5.18 (m, 1H), 5.06 (s, 2H), 4.61 (m, 1H), 4.35 (dd, 1H, J=5.2, 10.0 Hz), 4.29 (dd, 1H, J=5.6, 10.0 Hz), 4.20 (dd, 1H, J=2.0, 10.8 Hz), 3.84 (dd, 1H, J=3.2, 9.6 Hz), 1.88 (s, 3H), 1.86 (s, 3H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 155.8, 148.0, 138.9, 129.1, 128.5, 125.7, 118.5, 74.7, 74.0, 70.8, 68.5, 21.8, 15.7; IR (thin film) ν_{max} 3409, 3137, 2960, 2930, 2882, 2856, 1639, 1620, 1496, 1442, 1416, 1364, 1232, 1074, 1012, 882, 803, 739 cm^{-1}; LRMS $[M]^+$ calculated for $C_{16}H_{20}N_4O_3$: 316.4, found: 316.0; $[\alpha]_D^{23}=-54.5°$ (c=0.67, CH_2Cl_2).

4.2.3. 2-[4-(4-Aminooxymethyl-phenyl)-[1,2,3]triazol-1-yl]-(1*R*)-(3-methoxy-phenyl)-ethanol (acetone oxime of 30). Colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (s, 1H), 7.70 (s, 1H), 7.65 (d, 1H, J=8.0 Hz), 7.30 (m, 3H), 6.98 (m, 2H), 6.86 (m, 1H), 5.22 (dd, 1H, J=3.2, 8.8 Hz), 5.05 (s, 1H), 4.66 (dd, 1H, J=3.2, 14.0 Hz), 4.44 (dd, 1H, J=8.8, 13.6 Hz), 3.79 (s, 3H), 1.98 (s, 1H), 1.89 (s, 3H), 1.85 (s, 3H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 160.1, 155.7, 147.3, 141.9, 139.0, 130.4, 130.0, 128.9, 127.7, 125.1, 124.9, 121.4, 118.1, 114.1, 111.3, 75.0, 72.8, 57.5, 55.2, 21.8, 15.7; IR (thin film) ν_{max} 3305, 3141, 2923, 1601, 1489, 1458, 1434, 1367, 1266, 1073, 10489, 790, 699 cm^{-1}; LRMS $[M]^+$ calculated for $C_{21}H_{24}N_4O_3$: 380.4, found: 380.2; $[\alpha]_D^{23}=-24.4°$ (c=0.54, CH_2Cl_2).

4.2.4. (4*R*)-[4-(2-Aminooxymethyl-phenyl)-[1,2,3]triazol-1-yl]-tetrahydro-furan-(3*R*)-ol (acetone oxime of 31). Colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.88 (dd, 1H, J=1.2, 7.6 Hz), 7.41 (m, 3H), 5.20 (m, 1H), 5.04 (m, 2H), 4.61 (m, 1H), 4.36 (dd, 1H, J=5.2, 10.4 Hz), 4.24 (dd, 1H, J=5.6, 10.4 Hz), 4.18 (dd, 1H, J=3.2, 10.4 Hz), 3.83 (dd, 1H, J=3.2, 10.0 Hz), 1.98 (s, 1H), 1.88 (s, 3H), 1.84 (s, 3H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 156.4, 146.3, 134.1, 131.0, 130.3, 129.0, 128.8, 128.5, 121.5, 74.0, 73.97, 70.8, 68.3, 21.8, 15.7; IR (thin film) ν_{max} 3362, 2925, 2880, 1648, 1435, 1368, 1269, 1227, 1074, 914, 767 cm^{-1}; LRMS $[M]^+$ calculated for $C_{16}H_{20}N_4O_3$: 316.4, found: 316.0; $[\alpha]_D^{23}=-52.8°$ (c=0.59, CH_2Cl_2).

4.2.5. 1-[4-[(1*S*)-Aminooxy-ethyl)-[1,2,3]triazol-1-yl]-3-isopropoxy-propan-(2*R*)-ol (acetone oxime of 32). Colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (s, 1H), 5.37 (q, 1H, J=6.8 Hz), 4.52 (dd, 1H, J=3.6, 14.0 Hz), 4.39 (dd, 1H, J=6.4, 14.0 Hz), 4.13 (m, 1H), 3.56 (m, 1H), 3.44 (dd, 1H,



Scheme 11. Synthesis of propargylic amine-containing alkoxyamines. Reagents and conditions: (a) CH_2Cl_2, *p*-TsOH·H_2O, $(CH_2O)_n$. (b) CuBr, DMF, 50 °C. (c) NH_2NH_2, CH_2Cl_2.

J=5.2, 9.6 Hz), 3.34 (dd, 1H, J=6.0, 9.6 Hz), 1.83 (s, 6H), 1.63 (d, 3H, J=8.4 Hz), 1.13 (s, 3H), 1.12 (s, 3H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 155.2, 150.0, 122.8, 73.2, 72.4, 69.3, 68.7, 52.7, 21.9, 21.8, 19.9, 15.7; IR (thin film) ν_{max} 3333, 2972, 2924, 1457, 1371, 1262, 1130, 1078, 935, 668 cm^{-1}; LRMS $[M]^+$ calculated for $C_{13}H_{24}N_4O_3$: 284.4, found: 284.5; $[\alpha]_D^{23}$=+11.9° (c=0.55, CH_2Cl_2).

4.2.6. Representative procedure for synthesis of isoxazole-containing alkoxyamines (acetone oxime of 41). Cyclohexanecarboxaldehyde (21.6 mg, 0.193 mmol), hydroxylamine hydrochloride (26.7 mg, 0.385 mmol) were mixed with 1 mL CH_2Cl_2 in the presence of Et_3N (79 μL, 0.578 mmol). The reaction mixture was stirred for 1 h, diluted with CH_2Cl_2, and subsequently applied to an SLE cartridge (5.0 mL) containing 1 N HCl. After elution with 15 mL CH_2Cl_2, the resulting oxime solution was concentrated and reacted with NBS in CH_2Cl_2 for 3 h. The reaction mixture was transferred to a 5 mL reaction vessel on the Quest-210 synthesizer containing the alkyne resin (0.0385 mmol, see procedure for preparation of **33**) and $NaHCO_3$ (16.4 mg, 0.193 mmol). The reaction was agitated at 35 °C for 12 h to afford the corresponding isoxazole-containing resin. After filtration and resin washing with H_2O (2×3 mL), MeOH (1×3 mL), DMF (2×3 mL), THF (3×3 mL) and CH_2Cl_2 (3×3 mL), 2 mL CH_2Cl_2 and NH_2NH_2 (2.5 μL, 0.077 mmol) were added and the reaction was agitated for 1 h. The reaction was filtered, the resin washed with CH_2Cl_2, and the filtrate collected and evaporated to provide 7.8 mg (0.0374 mmol, 72.4%) of *O*-[(1*S*)-(3-cyclohexyl-isoxazol-5-yl)-ethyl]-hydroxylamine **41** as a colorless oil. **41** was treated with 2 mL acetone and then concentrated, and the resulting sample was subjected to HPLC-ELSD analysis using a Waters HPLC-MS system (XTerra®RP_8, 5 μm, 4.6×30 mm column) to determine the purity (>99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.98 (s, 1H), 5.27 (q, 1H, J=7.2 Hz), 2.70 (m, 1H), 1.96 (m, 2H), 1.86 (s, 3H), 1.84 (s, 3H), 1.75 (m, 3H), 1.38 (m, 5H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 172.9, 168.3, 156.1, 99.4, 72.9, 35.8, 31.9, 25.88, 25.77, 21.7, 18.8, 15.7; IR (thin film) ν_{max} 2985, 2929, 2854, 1603, 1450, 1423, 1371, 1343, 1269, 1155, 1087, 986, 933, 891, 803 cm^{-1}; LRMS $[M]^+$ calculated for $C_{14}H_{22}N_2O_2$: 250.3, found: 250.4; $[\alpha]_D^{23}$=−2.9° (c=0.28, CH_2Cl_2).

4.2.7. *O*-[4-(3-Phenyl-isoxazol-5-yl)-benzyl]-hydroxylamine (acetone oxime of 38). Colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (m, 4H), 7.46 (m, 5H), 6.80 (s, 1H), 5.10 (s, 2H), 1.91 (s, 3H), 1.87 (s, 3H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 170.4, 163.1, 155.9, 140.9, 130.1, 129.1, 129.0, 128.3, 127.5, 126.9, 126.7, 125.9, 97.4, 74.5, 21.8, 15.7; IR (thin film) ν_{max} 3106, 2922, 2856, 1620, 1566, 1503, 1463, 1367, 1074, 950, 816, 767, 671 cm^{-1}; LRMS $[M]^+$ calculated for $C_{19}H_{18}N_2O_2$: 306.4, found: 306.2.

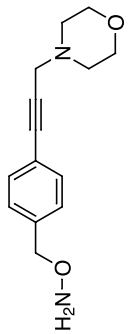
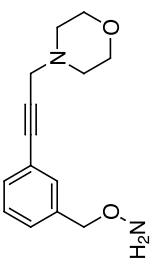
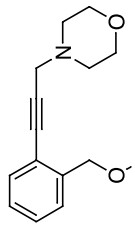
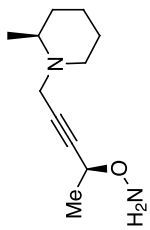
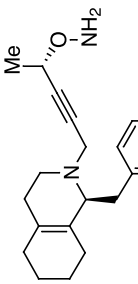
4.2.8. *O*-[3-(3-Phenyl-isoxazol-5-yl)-benzyl]-hydroxylamine (acetone oxime of 39). Pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) (spectrum of the major isomer reported) δ 7.85 (m, 2H), 7.84 (s, 1H), 7.76 (d, 1H, J=6.8 Hz), 7.46 (m, 5H), 6.82 (s, 1H), 5.12 (s, 2H), 1.91 (s, 3H), 1.87 (s, 3H); ^{13}C NMR (75.0 MHz, $CDCl_3$) (spectrum of the mixture of both isomers reported) δ 170.5, 163.1, 155.9, 155.8, 139.6, 138.7, 137.0, 130.1, 129.7, 129.1, 129.0, 128.8, 128.5, 128.4, 128.3, 127.5, 126.9, 125.2, 125.1, 121.4, 103.0, 97.6, 74.62, 74.56, 21.8, 15.7; IR (thin film) ν_{max} 3063, 2920, 2869, 1608, 1573, 1484, 1468, 1447, 1401, 1366, 1271, 1073, 1019, 921, 791, 767, 694 cm^{-1}; CIHRMS $[M]^+$ calculated for $C_{19}H_{18}N_2O_2$: 306.4, found: 306.2.

4.2.9. *O*-{(1*S*)-[3-(2,4,6-Trimethyl-phenyl)-isoxazol-5-yl]-ethyl}-hydroxylamine (acetone oxime of 40). Colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (s, 2H), 6.07 (s, 1H), 5.38 (q, 1H, J=6.8 Hz), 2.29 (s, 3H), 2.12 (s, 6H), 1.88 (s, 3H), 1.85 (s, 3H), 1.66 (d, 3H, J=7.2 Hz); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 173.5, 161.9, 156.1, 138.7, 137.3, 128.3, 102.75, 102.67, 72.9, 21.6, 21.0, 20.1, 18.7, 15.6; IR (thin film) ν_{max} 2986, 2923, 1613, 1445, 1373, 1268, 1090, 1030, 986, 933, 890, 671, 656 cm^{-1}; LRMS $[M]^+$ calculated for $C_{17}H_{22}N_2O_2$: 286.4, found: 286.6; $[\alpha]_D^{23}$=+12.1° (c=1.57, CH_2Cl_2).

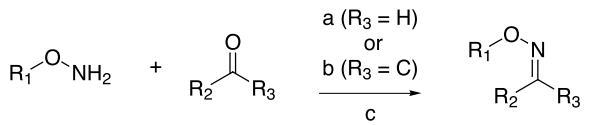
4.2.10. *O*-{(1*S*)-[3-(2,4-Dimethoxy-phenyl)-isoxazol-5-yl]-ethyl}-hydroxylamine (acetone oxime of 42). Pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, 1H, J=2.8 Hz), 6.92 (m, 2H), 6.67 (s, 1H), 5.36 (q, 1H, J=6.8 Hz), 3.82 (s, 3H), 3.79 (s, 3H), 1.87 (s, 3H), 1.84 (s, 3H), 1.64 (d, 3H, J=7.2 Hz); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 172.9, 159.8, 156.1, 153.7, 151.7, 118.6, 117.2, 113.6, 113.1, 102.9, 72.7, 56.1, 55.8, 21.7, 18.6, 15.7; IR (thin film) ν_{max} 2990, 2937, 1602, 1511, 1471, 1438, 1372, 1275, 1226, 1045, 857, 745, 669 cm^{-1}; LRMS $[M]^+$ calculated for $C_{16}H_{20}N_2O_4$: 304.3, found: 304.2; $[\alpha]_D^{23}$=+4.5° (c=1.33, CH_2Cl_2).

4.2.11. Representative procedure for synthesis of propargylic amine-containing alkoxyamines (acetone oxime of 50). (*R*)-(+)-1-(4-Methoxybenzyl)-1,2,3,4,5,6,7,8-octahydroisoquinolline (49.6 mg, 0.193 mmol) was mixed with

Table 3. Diverse propargylamine-containing alkoxyamines

Substrate					
	46	**47**	**48**	**49**	**50**
Yield (%)[a]	99	99	96	95	95
Purity (%)[b]	>99	>99	>99	>99	97

[a] Isolated yield based on the loading of the *N*-hydroxyphthalimide resin **1** resin (0.68 mmol/g).
[b] Purity determined by HPLC-ELSD analysis of the corresponding acetone oxime.



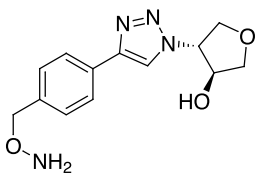
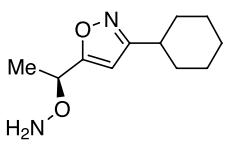
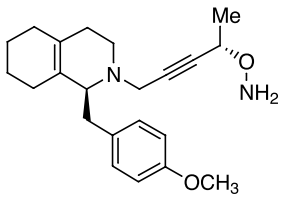
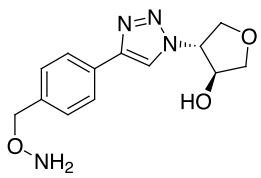
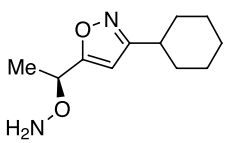
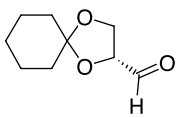
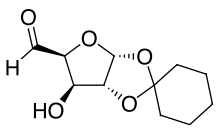
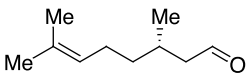
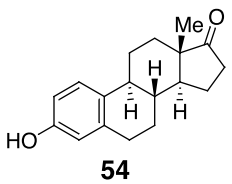
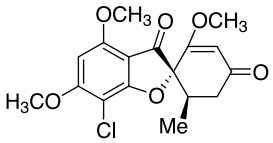
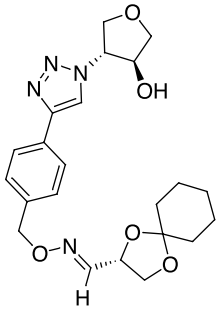
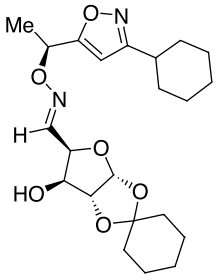
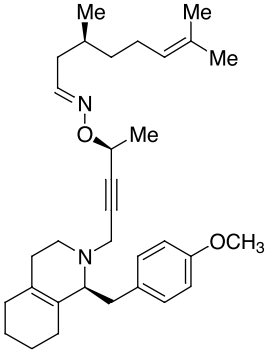
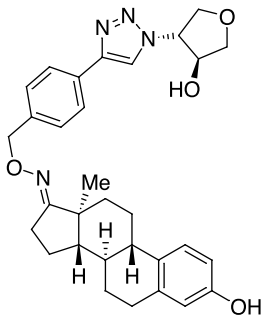
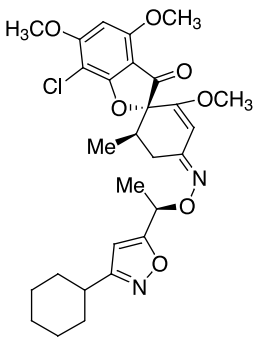
Scheme 12. Oxime formation. Reagents and conditions: (a) $ClCH_2CH_2Cl$, 120 °C, 150 W, 15 min. (b) HOAc, EtOAc, 150 °C, 150 W, 30 min. (c) PL-MIA, CH_2Cl_2, 4 h.

paraformaldehyde (9.2 mg, 0.29 mmol), *p*-TsOH·H_2O (36.7 mg, 0.193 mmol), and 1 mL CH_2Cl_2. The reaction mixture was stirred for 3 h and then concentrated. The resulting solid was mixed with 2 mL DMF and transferred to a 5 mL reaction vessel on the Quest-210 synthesizer containing the alkyne resin (0.0385 mmol, see procedure for preparation of **33**) and CuBr (2.7 mg, 0.0193 mmol, 50 mol%). The reaction was agitated at 50 °C for 12 h to afford the corresponding propargylic amine-containing resin. After filtration and resin washing with H_2O (2×3 mL), MeOH (1×3 mL), DMF (2×3 mL), MeOH (1×3 mL), 10% *N*,*N*,*N*′,*N*′-tetramethylethylenediamine in CH_2Cl_2 (2×3 mL), CH_2Cl_2 (1×3 mL), 5% AcOH in CH_2Cl_2 (2×3 mL), THF (3×3 mL) and CH_2Cl_2 (3×3 mL), 2 mL CH_2Cl_2 and NH_2NH_2 (2.5 μL, 0.077 mmol) was added and the reaction was agitated for 1 h. The reaction was filtered, the resin was washed with CH_2Cl_2, and the filtrate was collected and evaporated to provide 12.9 mg (0.0363 mmol, 70.7%) of *O*-{4-[(1*R*)-(4-methoxy-benzyl)-3,4,5,6,7,8-hexahydro-1*H*-isoquinolin-2-yl]-(1*S*)-methyl-but-2-ynyl}-hydroxylamine **50** as a pink oil. **50** was treated with 2 mL acetone, concentrated, and the resulting sample subjected to HPLC-ELSD analysis using a Waters HPLC-MS system (XTerra®RP_8, 5 μm, 4.6×30 mm column) to determine the purity (97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (d, 2H, *J*=8.4 Hz), 6.77 (d, 2H, *J*=8.4 Hz), 4.81 (q, 1H, *J*=6.7 Hz), 3.75 (s, 3H), 3.51 (dd, 2H, *J*=16.4, 38.0 Hz), 3.26 (s, 1H), 2.90 (m, 1H), 2.78 (m, 3H), 1.90 (m, 2H), 1.85 (s, 3H), 1.84 (s, 3H), 1.64 (m, 8H), 1.44 (d, 3H, *J*=6.8 Hz); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 157.8, 155.4, 132.6, 130.2, 129.1, 127.7, 113, 68.4, 62.3, 55.1, 45.3, 43.5, 35.7, 30.0, 28.1, 27.9, 23.0, 22.7, 21.8, 21.1, 15.7; IR (thin film) ν_{max} 2086, 2928, 2832, 1613, 1512, 1440, 1368, 1326, 1247, 1176, 1072, 1038, 931, 824, 659 cm^{-1}; CIHRMS $[M]^+$ calculated for $C_{25}H_{34}N_2O_2$: 394.5, found: 394.3; $[\alpha]_D^{23}=-4.3°$ (*c*=0.55, CH_2Cl_2).

4.2.12. *O*-[4-(3-Morpholin-4-yl-prop-1-ynyl)-benzyl]-hydroxylamine (acetone oxime of 46). Colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, 2H, *J*=8.0 Hz), 7.27 (d, 2H, *J*=8.4 Hz), 5.02 (s, 2H), 3.77 (t, 4H, *J*=4.8 Hz), 3.50 (s, 2H), 2.65 (t, 4H, *J*=3.6 Hz), 1.86 (s, 3H), 1.85 (s, 3H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 155.7, 138.7, 131.7, 127.7, 122.1, 85.7, 83.6, 74.7, 66.7, 52.2, 48.0, 21.8, 15.7; IR (thin film) ν_{max} 2922, 2855, 2814, 1510, 1454, 1367, 1117, 1072, 1012, 828, 669, 655 cm^{-1}; LRMS $[M]^+$ calculated for $C_{17}H_{22}N_2O_2$: 286.4, found: 285.9.

4.2.13. *O*-[3-(3-Morpholin-4-yl-prop-1-ynyl)-benzyl]-hydroxylamine (acetone oxime of 47). Colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (m, 2H), 7.29 (m, 1H), 7.20 (m, 1H), 5.21 (s, 2H), 3.77 (t, 4H, *J*=4.4 Hz), 3.56 (s, 2H), 2.67 (t, 4H, *J*=4.4 Hz), 1.90 (s, 3H), 1.86 (s, 3H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 155.6, 140.2, 132.4, 128.4, 127.7, 127.3, 121.5, 88.2, 83.5, 73.4, 66.7, 52.1, 48.0, 21.8, 15.7;

Table 4. Synthesis of complex oximes

Alkoxyamines					
	29	**41**	**50**	**29**	**41**
Aldehyde/ketone					
	51	**52**	**53**	**54**	**55**
Products					
	57[a]	**58**[b]	**59**[c]	**60**	**61**[b]
Yield (%)	95	96	93	86	89
Purity (%)[d]	98	99	99	97	98

[a] Isolated as a 2:1 (*E*:*Z*) mixture of oximes, as determined by ^{1}H NMR (see Ref. 1a).
[b] Isolated as a 1:1 (*E*:*Z*) mixture of oximes, as determined by ^{1}H NMR (see Ref. 1a).
[c] Isolated as a 7:1 (*E*:*Z*) mixture of oximes, as determined by ^{1}H NMR (see Ref. 1a).
[d] Purity determined by HPLC-ELSD analysis.

IR (thin film) ν_{max} 2958, 2921, 2855, 2815, 1484, 1452, 1366, 1117, 1072, 1011, 761, 666 cm^{-1}; CIHRMS $[M]^+$ calculated for $C_{17}H_{22}N_2O_2$: 286.4, found: 285.9.

4.2.14. *O*-[2-(3-Morpholin-4-yl-prop-1-ynyl)-benzyl]-hydroxylamine (acetone oxime of 48). Colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.40 (s, 1H), 7.29 (m, 3H), 5.00 (s, 2H), 3.78 (t, 4H, J=4.0 Hz), 3.51 (s, 2H), 2.65 (m, 4H), 1.87 (s, 3H), 1.85 (s, 3H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 155.6, 138.7, 131.1, 131.0, 128.3, 127.8, 122.9, 85.8, 83.6, 74.6, 66.7, 52.3, 48.0, 21.8, 15.7; IR (thin film) ν_{max} 2956, 2924, 2855, 1484, 1453, 1367, 1267, 1118, 1073, 1006, 797, 692, 671 cm^{-1}; LRMS $[M]^+$ calculated for $C_{17}H_{22}N_2O_2$: 286.4, found: 285.9.

4.2.15. *O*-{(1*S*)-Methyl-4-[(2*S*)-methyl-piperidin-1-yl]-but-2-ynyl}-hydroxylamine (acetone oxime of 49). Colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 4.83 (q, 1H, J=5.3 Hz), 3.69 (dd, 1H, J=2.0, 15.2 Hz), 3.36 (par obsc, 1H), 2.77 (m, 1H), 2.44 (m, 1H), 2.36 (m, 1H), 1.86 (s, 3H), 1.84 (s, 3H), 1.61 (m, 4H), 1.46 (d, 3H, J=6.8 Hz), 1.26 (m, 2H), 1.06 (d, 3H, J=6.0 Hz); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 155.6, 86.0, 78.4, 68.3, 54.7, 53.2, 43.5, 34.5, 26.0, 24.4, 21.8, 21.2, 19.8, 15.7; IR (thin film) ν_{max} 3283, 2931, 2855, 2801, 1650, 1544, 1440, 1372, 1322, 1157, 1074, 990, 932, 892, 656 cm^{-1}; LRMS $[M]^+$ calculated for $C_{14}H_{24}N_2O$: 236.4, found: 236.0; $[\alpha]_D^{23}$=+6.0° (c=0.61, CH_2Cl_2).

4.2.16. Representative procedure for the synthesis of oxime ethers from aldehydes. To a standard microwave reaction vessel (CEM Corp.) equipped with a stir bar was charged (*S*)-citronellal **53** (0.0044 mL, 0.024 mmol), *O*-{4-[(3*R*)-(4-methoxy-benzyl)-3,4,5,6,7,8-hexahydro-1*H*-isoquinolin-2-yl]-(1*S*)-methyl-but-2-ynyl}-hydroxylamine **50**, (0.0104 g, 0.0293 mmol, 1.2 equiv.), and dichloroethane (0.075 mL, 0.3 M). The mixture was heated under microwave irradiation for 15 min at 120 °C (300 W). The reaction mixture was cooled to rt and charged with PL-MIA resin (0.0063 g, 0.0162 mmol, 3 equiv.) and stirred at room temperature for 4 h. The resin was filtered and rinsed with CH_2Cl_2 (3×1 mL). The filtrate was concentrated under reduced pressure to yield (3*S*),7-dimethyl-oct-6-enal *O*-{4-[(1*R*)-(4-methoxy-benzyl)-3,4,5,6,7,8-hexahydro-1*H*-isoquinolin-2-yl]-(1*S*)-methyl-but-2-ynyl}-oxime **59** (0.0109 g, 0.0222 mmol, 93% yield) as a pink oil (7:1 mixture of *E*:*Z* oxime ethers). The product was subjected to an HPLC-ELSD analysis using a Waters HPLC-MS system (XTerra®RP_8, 5 μm, 4.6×30 mm column) to determine the purity (99%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.38 (t, 1H, J=6.6 Hz, *E*), 7.16 (d, 2H, J=8.6 Hz), 6.76 (d, 2H, J=8.6 Hz), 6.69 (t, 1H, J=5.4 Hz, *Z*), 5.07–5.03 (m, 1H), 4.84–4.79 (m, 1H), 3.76 (s, 3H), 3.44 (dd, 2H, J=17.0, 42.4 Hz), 3.21 (m, 1H), 2.89–2.87 (m, 1H), 2.76 (m, 3H), 2.22–2.15 (m, 1H), 2.06–1.80 (m, 6H), 1.66 (s, 3H), 1.58, (s, 3H), 1.43 (d, 3H, J=6.6 Hz), 1.39–1.27 (m, 2H), 1.23–1.14 (m, 3H), 0.90 (d, 3H, J=6.6 Hz); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 151.1, 130.6, 130.2, 127.8, 124.4, 113.4, 77.2, 68.6, 62.3, 55.1, 45.3, 43.4, 36.7, 36.6, 36.3, 35.7, 32.6, 30.8, 30.5, 30.1, 29.6, 28.1, 25.6, 25.3, 23.0, 22.7, 21.0, 19.6, 19.3, 17.5; IR (thin film) ν_{max} 2926, 2834, 2739, 1613, 1512, 1458, 1376, 1327, 1247, 1177, 1072, 1040, 935, 830, 775, 661; LRMS $[M]^+$ calculated for $C_{32}H_{46}N_2O_2$: 490.4, found: 490.6; $[\alpha]_D^{23}$=+4.2° (c=0.28, CH_2Cl_2).

4.2.17. 1,4-Dioxa-spiro[4.5]decane-2-carbaldehyde *O*-{4-[1-[(4*R*)-hydroxy-tetrahydro-furan-(3*R*)-yl]-1*H*-[1,2,3]triazol-4-yl]-benzyl}-oxime 57. Pale yellow oil isolated as a 2:1 mixture of *E*:*Z* oxime ethers. ^1H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.78 (d, 2H, J=7.9 Hz), 7.39 (m, 2H), 7.36 (d, 1H, J=8.2 Hz, *E*), 6.93 (d, 1H, J=4.3 Hz, *Z*), 5.14 (m, 1H), 5.09 (s, 2H, *E*), 5.08 (s, 2H, *Z*), 4.69 (m, 1H), 4.61 (dd, 1H, J=6.3, 12.9 Hz), 4.38 (dd, 1H, J=5.4, 10.4 Hz), 4.30 (dd, 1H, J=5.4, 10.1 Hz), 4.23 (dd, 1H, J=2.5, 10.4 Hz, 1H), 4.13 (dd, 1H, J=6.6, 8.6 Hz, *E*), 3.86–3.83 (m, 2H), 3.72 (dd, 1H, J=6.8, 8.4 Hz, *Z*), 1.59 (m, 8H), 1.38 (m, 2H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 153.0, 149.3, 147.9, 137.8, 137.6, 129.6, 128.9, 128.7, 128.4, 125.8, 125.7, 118.5, 110.9, 110.4, 77.1, 76.6, 75.9, 75.7, 74.0, 72.8, 70.8, 70.5, 68.5, 67.6, 67.0, 60.4, 36.0, 35.5, 34.8, 34.7, 29.6, 25.7, 24.9, 23.76, 23.72, 23.68, 20.9, 14.0; IR (thin film) ν_{max} 3410, 3137, 2933, 2856, 1447, 1366, 1232, 1164, 1110, 1076, 1041, 1014, 975, 930, 803, 696, 655 cm^{-1}; LRMS $[M]^+$ calculated for $C_{22}H_{28}N_4O_5$: 428.2, found: 428.2; $[\alpha]_D^{23}$=−20.2° (c=0.67, CH_2Cl_2).

4.2.18. 1,2-*O*-Cyclohexylidene-α-D-xylopentodialdo-1,4-furanose *O*-[(1*S*)-(3-cyclohexyl-isoxazol-5-yl)-ethyl]-oxime 58. Pale yellow oil isolated as a 1:1 mixture of *E*:*Z* oxime ethers. ^1H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, 1H, J=5.3 Hz, *E*), 6.85 (d, 1H, J=3.0 Hz, *Z*), 6.0 (s, 1H), 5.97 (d, 1H, J=3.6 Hz), 5.37 (dd, 1H, J=6.9, 13.7 Hz, *Z*), 5.31 (dd, 1H, J=6.9, 13.7 Hz, *E*), 5.01 (t, 1H, J=3.0 Hz, *Z*), 4.67 (m, 1H), 4.55 (t, 1H, J=3.3 Hz), 4.34 (d, 1H J=2.6 Hz, *E*), 2.74–2.67 (m, 1H), 1.96–1.94 (m, 2H), 1.81–1.78 (m, 2H), 1.72–1.64 (m, 4H), 1.60 (dd, 3H, J=6.9, 8.2 Hz), 1.57–1.52 (m, 4H), 1.49–1.31 (m, 6H), 1.28–1.23 (m, 2H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 172.2, 172.0, 168.9, 168.8, 150.7, 148.2, 113.1, 113.0, 105.1, 104.6, 100.7, 100.4, 85.0, 84.9, 78.4, 78.0, 76.7, 76.0, 74.3, 74.1, 36.8, 36.7, 36.1, 35.9, 32.3, 32.2, 32.1, 26.2, 26.1, 26.0, 25.1, 24.2, 24.1, 23.8, 18.8, 18.7; IR (thin film) ν_{max} 3415, 2934, 2865, 1604, 1451, 1371, 1341, 1291, 1232, 1076, 1019, 940, 867, 808, 759, 696, 674 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{22}H_{32}N_2O_6$: 420.2, found: 420.2; $[\alpha]_D^{23}$=−11.1° (c=1.22, CH_2Cl_2).

4.2.19. Representative procedure for synthesis of oxime ethers from ketones. To a standard microwave reaction vessel (CEM Corp.) equipped with a stir bar was charged estrone **54** (0.0079 g, 0.029 mmol), *O*-alkylhydroxylamine **30**, (0.0097 g, 0.035 mmol, 1.2 equiv.), acetic acid (0.025 mL, 0.44 mmol, 15 equiv.), and ethyl acetate (0.50 mL, 0.06 M). The mixture was heated under microwave irradiation for 30 min at 150 °C (300 W). The cooled reaction mixture was concentrated under reduced pressure. The mixture was re-suspended in dichloromethane (0.20 mL), and charged with PL-MIA (0.0071 g, 0.018 mmol, 3 equiv) and stirred at room temperature for 4 h. The resin was filtered and rinsed with CH_2Cl_2 (3×1 mL). The filtrate was concentrated under reduced pressure to yield 3-hydroxylestra-1,3,5(10)-triene-17-one *O*-{4-[1-[(4*R*)-hydroxy-tetrahydro-furan-(3*R*)-yl]-1*H*-[1,2,3]-triazol-4-yl]-benzyl}-oxime **60** (0.0133 g, 0.025 mmol, 86% yield) as a clear, yellow oil. The product was subjected to an HPLC-ELSD analysis using a Waters HPLC-MS system (XTerra®RP_8, 5 μm, 4.6×30 mm column) to determine the purity (97%). ^1H NMR (400 MHz, $CDCl_3$)

δ 7.80 (s, 1H), 7.68 (d, 2H, J=8.2 Hz), 7.30 (d, 2H, J=7.9 Hz), 6.99 (d, 1H, J=8.6 Hz), 6.52 (dd, 1H, J=2.6, 8.6 Hz), 6.46 (s, 1H), 5.01 (m, 1H), 4.97 (s, 2H), 4.47 (m, 1H), 4.25 (dd, 1H, J=5.6, 10.2 Hz), 4.16–4.11 (m, 2H), 3.71 (dd, 1H, J=3.3, 9.9 Hz), 2.73–2.69 (m, 2H), 2.48–2.38 (m, 2H), 2.24–2.20 (m, 1H), 2.14–2.09 (m, 1H), 1.95–1.90 (m, 1H), 1.81–1.75 (m, 2H), 1.52–1.22 (m, 6H), 1.16–1.12 (m, 2H), 0.82 (s, 3H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 171.5, 153.7, 138.9, 138.1, 132.3, 129.1, 128.6, 128.4, 128.3, 126.5, 125.6, 118.5, 115.3, 112.8, 77.1, 74.9, 74.1, 70.1, 68.4, 52.8, 44.3, 43.8, 38.0, 34.0, 29.6, 29.4, 27.1, 26.0, 22.9, 17.2; IR (thin film) ν_{max} 3314, 3147, 3056, 2927, 2868, 1732, 1652, 1612, 1583, 1501, 1453, 1418, 1371, 1285, 1247, 1232, 1099, 1078, 1048, 1015, 983, 915, 853, 736, 667 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{31}H_{36}N_4O_4$: 528.3, found: 528.5; $[\alpha]_D^{23}=-3.8°$ (c=0.16, CH_2Cl_2).

4.2.20. (2*S*)-*trans*-7-Chloro-2′,4,6-trimethoxy-(6′*S*)-methyl spiro(benzofuran-2[3*H*], 1′-[2]cyclohexene)-3,4′-dione-4′ *O*-[(1*S*)-(3-cyclohexyl-isoxazol-5-yl)-ethyl]-oxime 61. Pale yellow oil isolated as a 1:1 mixture of *E*:*Z* oxime ethers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.15 (s, 1H, *Z*), 6.08 (s, 1H), 6.01 (s, 1H), 5.56 (s, 1H, *E*), 5.28 (m, 1H), 3.99 (s, 3H), 3.94 (s, 3H), 3.58 (s, 3H, *E*), 3.51 (s, 3H, *Z*), 3.03 (dd, 1H, J=4.9, 16.8 Hz, *Z*), 2.92 (dd, 1H, J=13.2, 15.0 Hz, *E*), 2.74–2.65 (m, 1H), 2.64 (d, 1H, J=16.8 Hz, *Z*), 2.58–2.50 (m, 1H), 2.36 (dd, 1H, J=4.1, 15.0 Hz, *E*), 1.95 (m, 2H), 1.81–1.68 (m, 3H), 1.61 (d, 3H, J=6.6 Hz), 1.49–1.25 (m, 5H), 0.91 (d, 3H, J=6.3 Hz); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 194.3, 194.2, 172.7, 172.6, 169.6, 168.4, 164.5, 161.4, 158.8, 157.6, 157.5, 155.3, 152.0, 105.6, 99.7, 99.6, 99.0, 97.1, 93.2, 91.4, 91.3, 89.2, 89.1, 73.5, 73.4, 56.9, 56.3, 56.1, 55.9, 36.4, 35.8, 35.2, 31.9, 31.0, 29.6, 26.2, 25.9, 25.8, 18.7, 18.6, 14.2, 14.1; IR (thin film) ν_{max} 2930, 2853, 1707, 1613, 1590, 1466, 1351, 1220, 1141, 1099, 949, 912, 881, 733 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{28}H_{33}ClN_2O_7$: 544.2, found: 544.1; $[\alpha]_D^{23}=+85.6°$ (c=0.76, CH_2Cl_2).

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Solid-phase synthesis of lamellarins Q and O

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Abstract—An efficient solid phase synthesis of the pyrrole-based alkaloids lamellarins Q and O using Merrifield resin and *N*-protected methyl 3,4-dibromopyrrole-2-carboxylate as a scaffold is described.

1. Introduction

An important group of marine natural compounds including lamellarins,[1] lukianols, ningalins,[2] and polycytones[3] have a pyrrole ring as a core component of their skeleton. These compounds are important on the basis of their biological activities and the novelty of their structures, which are without precedent. Lamellarins and related compounds have been isolated from invertebrates such as the prosobranch mollusc *Lamellaria* sp., ascidians such as *Didemnum chartaceum* and the sponge *Dendrilla Cactos* collected from different sea areas. The structures of these natural products are related because they probably have a common biogenetic origin. The common structural feature is a pyrrole ring substituted at positions 3 and 4 by polyhydroxy- or methoxyphenyl groups. Lamellarins Q and O (**1**, **2**) are the simplest compounds in this family. The structure is present in more complex molecules such as polycytone A (**3**), which contains a symmetrically substituted pyrrole, and lukianol A (**4**) and ningaline A (**5**), in which new rings condensed to pyrrole are present due to lactonisation processes. Lamellarin A (**6**) possesses the most complex structure and is an example of a pentacyclic lamellarin that is characterised by a lactone and an isoquinoline both condensed to the pyrrole ring. This group of pentacyclic compounds contains the largest number of related natural products.

A considerable number of these natural products possess important cytotoxic activities (Fig. 1). For example, lukianol A exhibits activity against a cell line derived from human epidermatoid carcinoma,[1c] polycytone A inhibits the growth of SV 40 transformed fibroblast[3a] at concentrations of 10 $\mu g\ ml^{-1}$, lamellarins O and P demonstrated antibiotic activity,[1c] lamellarins D and C caused inhibition of cell division[1a] and lamellarin N tested by the NCI in a 60 cell-line panel showed selectivity towards melanoma cell lines.[1f] In addition, a select set of lamellarins exhibit equally potent cytotoxic activity against multidrug-resistant (MDR) cell lines arising over expression of P-glycoprotein and/or reverse MDR at noncyctotoxic concentrations, resensitising the resistant cell lines to conventional therapeutic agents.[4f]

A number of synthetic routes have been described for lamellarins,[4] lukianols,[4a,b,f,5] and ningalins[4f,6] but only one has been developed using the solid-phase approach.[7] Solid-phase combinatorial synthesis is one of the most useful techniques for the preparation of small libraries. This technique provides rapid access to larger collections of products that can possess great diversity and may incorporate optimised physical and pharmacological properties associated with certain structures.

Palladium-catalysed cross-coupling reactions are good methods for the preparation of bisaryl or heteroaryl derivatives. Our previous experience in Pd-catalysed heteroaryl coupling reactions[8] encouraged us to use this methodology for the solid phase preparation of the structurally least complex components of the group of compounds under investigation, namely lamellarins Q and O. The developed methodology will be used in the preparation of compound libraries in which elements of diversity would be introduced in the aromatic substituents at positions 3 and 4 and on the nitrogen of the pyrrole ring. For this reason it was important to develop a synthetic procedure that would be compatible with the sequential introduction of

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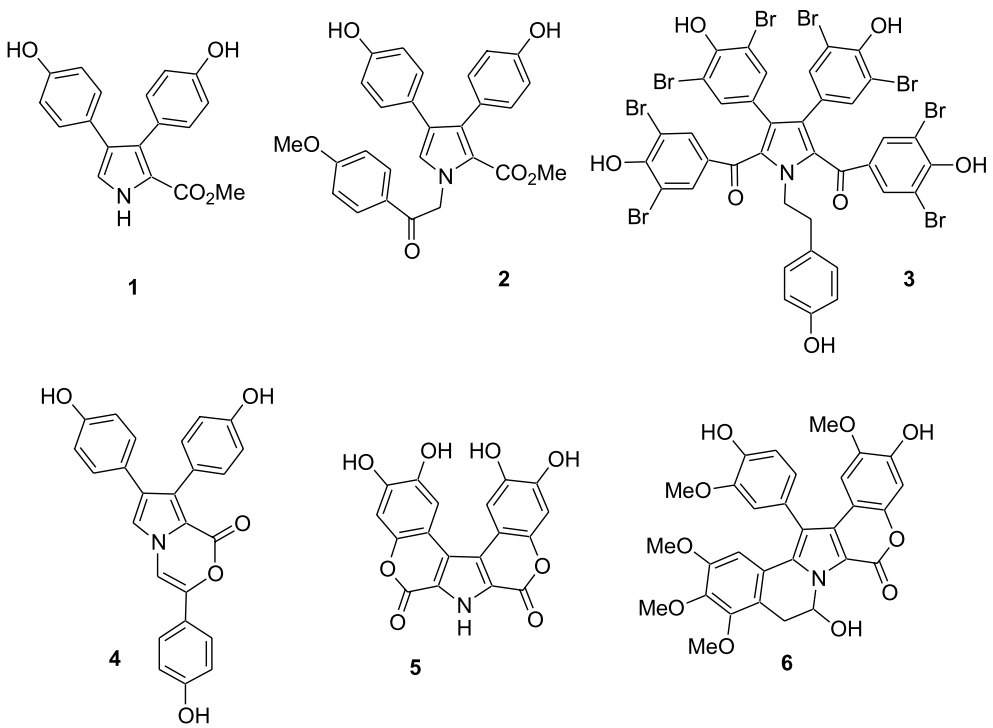


Figure 1.

these aromatic rings into an appropriately functionalised pyrrole ring.

We describe here a solid phase synthesis of lamellarins Q and O using methyl *N*-(triisopropylsilyl)-3,4-dibromo-pyrrole-2-carboxylate (**7a**) as a scaffold. Banwell et al.[4b] used the dibromopyrrole **7a** in an elegant convergent synthesis of several compounds from this family of marine alkaloids. As shown in Figure 2, compound **7** can be attached to the resin at three different positions that could be potentially useful for the preparation of related libraries using coupling methodologies in solid-phase synthesis.

The first strategy (a) involved linking the dibromopyrrole to the resin through the nitrogen to give **8**. However, this strategy was ruled out because it suffers from a serious limitation in that it lacks the flexibility required for the introduction of a nitrogen substituent and therefore loses one avenue to introduce diversity.

The results obtained on linking compounds **9** and **10** to a polystyrene (Merrifield) resin are now described. The formation of an ester linkage between the acid **7b** and hydroxymethyl functionalized Merrifield resin, as in compound **9** (strategy b), was tested for the sequential introduction of three elements of diversity into the pyrrole. Strategy c, on the other hand, involves compound **10** and requires an appropriately substituted iodophenol to be attached to a chloro-functionalised Merrifield resin. A

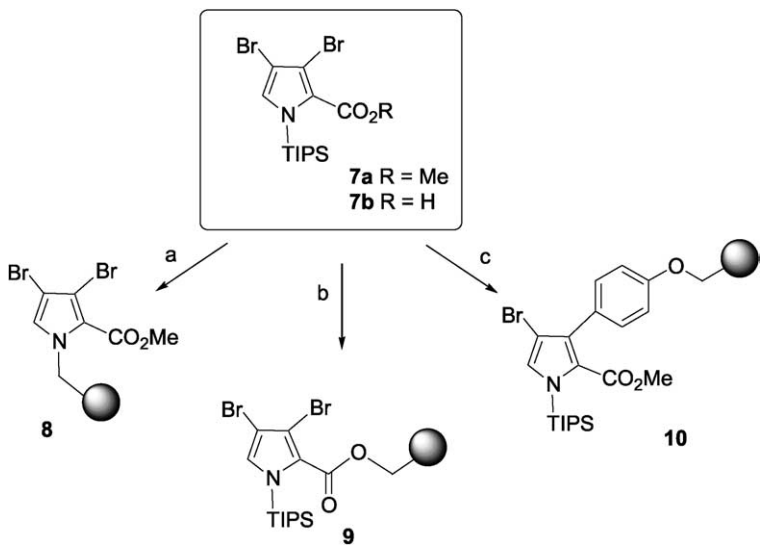


Figure 2.

subsequent Pd-catalysed cross-coupling reaction with a derivative of **7a** would then be used to introduce the scaffold.

2. Results and discussion

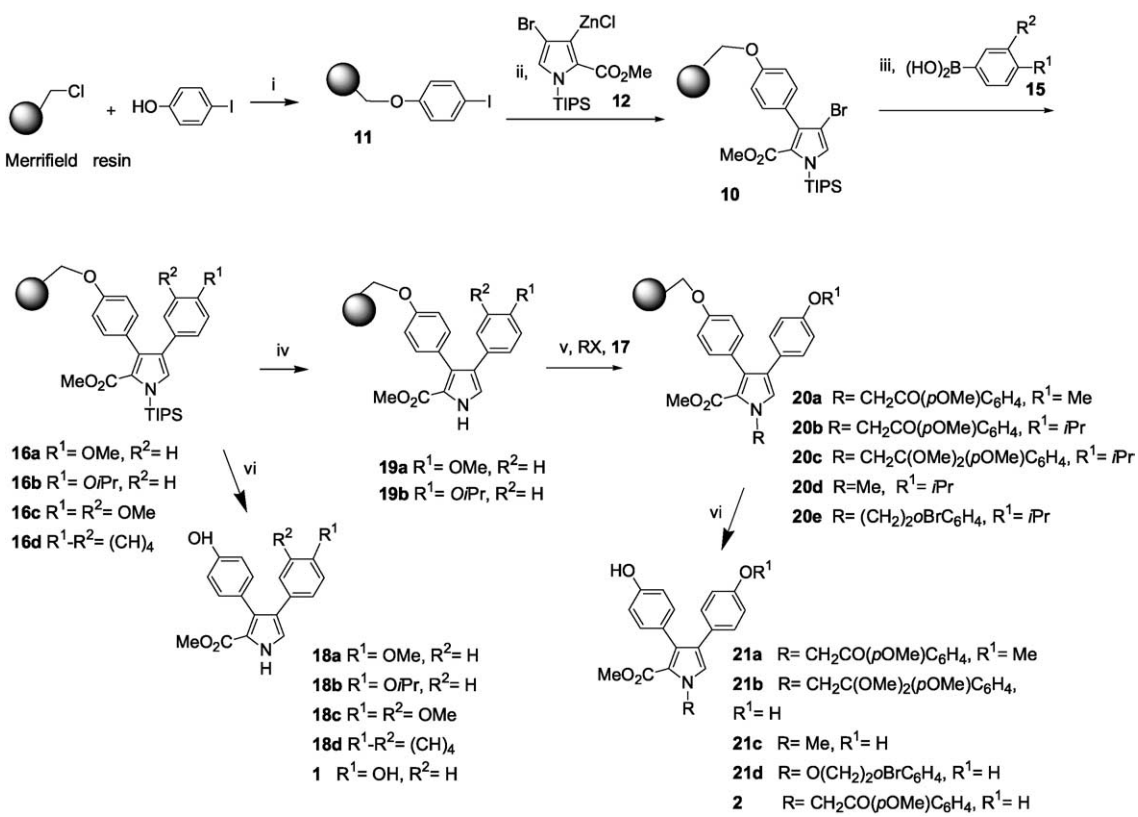
The anchored pyrrole **9** was obtained by reaction of the acid **7b** and hydroxy-functionalised Merrifield resin using DIPCDI as an activating agent and DMF as the solvent. Formation of **9** was confirmed by performing a cleavage reaction and characterising the resulting methyl 3,4-dibromopyrrole-2-carboxylate. Several experiments were performed to assess the regioselective formation of a zinc derivative of **9**, using *n*BuLi and $ZnCl_2$, followed by a Pd-catalysed cross-coupling reaction with *p*-iodoanisole. However, all attempts in this direction were unsuccessful. In all cases the same product, methyl 3,4-dibromopyrrole-2-carboxylate, was obtained after cleavage of the crude coupling reaction product. This result indicates that the bromo-lithium interchange, which occurs prior to formation of the zinc derivative, did not take place. It seems reasonable that this failure is due to steric hindrance caused by the resin being attached close to the reaction site.

As an alternative, strategy c was investigated using compound **10**. The attachment of the *p*-iodophenol to the chloro-functionalised Merrifield resin was achieved under basic conditions (Scheme 1).

4-Iodophenol was attached to the resin under basic conditions using the phenoxy anion, which displaces the Cl of the resin.[9] Treatment with NaOMe was also carried out in order to cap any reactive residual chloromethyl groups.

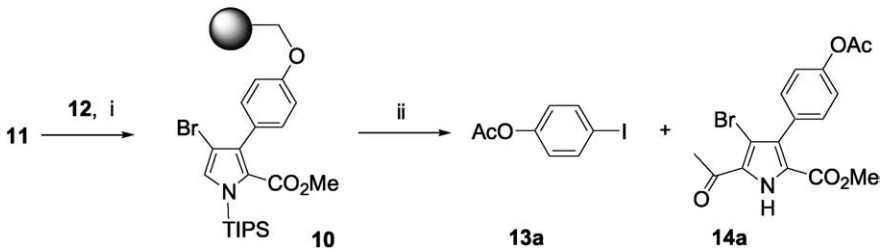
The methyl ester **7a**[10] was used for the chemoselective halogen–metal interchange and preparation of zinc derivative **12**. Compound **12** was obtained by selective *ortho*-directed bromine/lithium interchange at position 3 by treatment of **7a** with *n*-BuLi at low temperature followed by metal–metal interchange using zinc chloride. The organometallic compound **12** was employed in a Pd(0)-catalysed Negishi cross-coupling reaction with the resin-bound iodophenol **11**.

Several experiments were carried out in order to find the optimal conditions for obtaining **10** and the results of these are shown in Table 1. In all the experiments $Pd(PPh_3)_4$ was used as the catalyst and THF as the solvent. Two parameters were changed in an attempt to improve the yield of the coupling reaction; the relative proportion of the reagents and the reaction time. Each reaction was assessed by performing a cleavage reaction using $ZnBr_2$ and acetyl bromide in DCM and the ^1H NMR spectra of the crude product were evaluated. The cleavage using $ZnBr_2$ and AcBr produced several simultaneous reactions in addition to the desired ether cleavage; *O*-acetylation of the resulting phenol occurred along with *N*-desilylation of the pyrrole and acylation at position 5 of the pyrrole. Thus, under these cleavage conditions *O*-acetyl iodophenol **13a**—corresponding to the unreacted starting resin—and



Scheme 1. Reagents: (i) NaOMe, dry DMF, N_2, 80 °C, 24 h; (ii) $Pd(PPh_3)_4$, dry THF, N_2, rt, 24 h; (iii) aq. 2 M Na_2CO_3, $Pd(PPh_3)_4$, dioxane, reflux, 21–48 h; (iv) NH_4F, DCM/MeOH (1:1), reflux, 6 h; (v) NaH (or LDA), dry THF, N_2, −78 °C, 24 h; or 18-crown-6 (2.5 M in DMF), microwave, 100 °C, 30–40 W, 2 min; (vi) $AlCl_3$, dry DCM, rt, 3 h.

Table 1. Solid-phase cross-coupling reaction between the anchored iodophenol **11** and the zinc derivative **12**



Cross-coupling conditions			Products[a]
Entry	**12/11** (equiv.)	Time (h)	**13a/14a**
1	2.5	21	33:66
2	3	18	50:50
3	5	18	50:50
4	10	18	15:85
5	10	24	0:100

Reagents: (i) 15 mol% $Pd(PPh_3)_4$, dry THF, rt; (ii) AcBr (40 equiv.), $ZnBr_2$ (3.5 equiv.), dry DCM.
[a] Proportion of unreacted iodine with respect to the cross-coupling product calculated from the ^1H NMR spectra of the crude cleaved material.

the diacetylarylpyrrole **14a** were obtained. The relative integrations of the doublets at 6.86 and 7.17 ppm, due to the aromatic protons *ortho* to the acetate groups in each compound, indicate the proportion of compound **13a** to **14a**. The anchored arylpyrrole **10**[11] was obtained in quantitative yield (entry 5, Table 1) by treatment of resin **11** with 10 equiv. of **12** at rt for 24 h.

It was envisaged that bisarylpyrrole **16a** would be prepared from **10** by a new Pd(0)-catalysed cross-coupling reaction. Two different alternative procedures could be followed for the formation of the second aryl–pyrrole bond. The first would involve the preparation of the organometallic compound on the resin through a halogen–metal interchange of the bromo-substituent at position 4 of **10** and the second approach would employ an organometallic aryl compound for the cross-coupling reaction with the bromo-containing pyrrole **10** anchored on the resin.

Preparation of the zinc derivative of **10** by bromine/lithium interchange with *n*-BuLi followed by treatment with $ZnCl_2$ and a subsequent coupling reaction with 4-iodoanisole was tried under different catalytic conditions (entries 1–3, Table 2). Unfortunately, all the experiments with zinc derivatives of **10** produced, after cleavage, a crude material that was difficult to manipulate and did not contain either the product corresponding to the coupling or the starting material.[12]

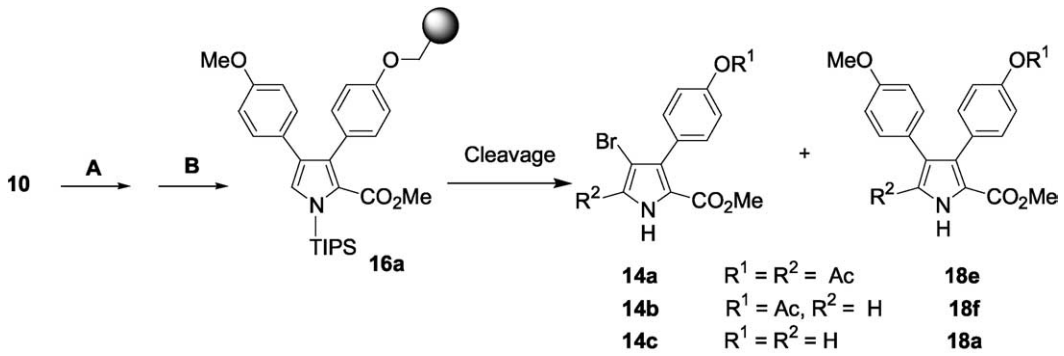
The second option for aryl–pyrrole bond formation was assessed and Suzuki[13] conditions were tried with resin **10**.[14] The different conditions tested for the Suzuki cross-coupling reaction between **10** and 4-methoxyphenylboronic acid are shown in Table 2 (entries 4–10).

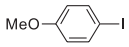
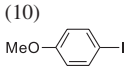
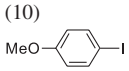
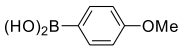
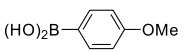
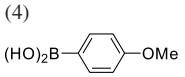
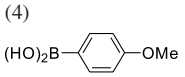
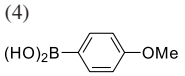
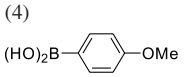
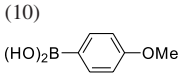
Formation of bisarylpyrrole **16a** was confirmed by gel phase nuclear magnetic resonance (GP ^{13}C NMR). The signal of the methoxy group at 67.0 ppm was indicative of the introduction of the new phenyl ring into the system. The amount of **16a** produced was determined after cleavage from the resin. Cleavage with $ZnBr_2$ and AcBr was used in entries 4–6, Table 2.[15] Under the same reaction conditions it was found that dioxane (entry 8) and dimethoxyethane (DME) (entry 7) gave better yields of **18** than THF (entry 4) as a solvent. Dioxane was the solvent of choice after comparison of yields obtained with these two solvents. $AlCl_3$ in DCM was used as the cleavage reagent (entries 7–9) to avoid the formation of acylated products **14b** and **18e**, which would be produced with $ZnBr_2$ and AcBr. Both cleavage reagents (entries 6 and 7) afforded the same yield but $AlCl_3$ has the advantage of producing cleaner cleavage products without side-reactions such as acetylation. Increasing the amount of boronic acid with respect to resin **10** (entry 8) produced only a small change in the yield, but an increase in the reaction time under these conditions to 21 h improved the yield to 84% (entry 10).

The same reaction conditions were applied to the synthesis of bisarylpyrroles **16b**-**16d**[16] using for the second cross-coupling reaction the appropriate boronic acid **15b**-**d**. Preparation of lamellarins Q and O requires the use of a *p*-hydroxyphenylboronic acid with a suitable hydroxy-protecting group for the introduction of the second aryl substituent. *p*-Isopropoxyphenylboronic acid was used for the cross-coupling reaction with bromide **10** and similar results were obtained as described above.[17] GP ^{13}C NMR was used to assess the success of the cross-coupling reaction, with the signals due to the isopropoxy group at 22.1 and 69.6 ppm (methyl and CH, respectively) confirming the formation of **16b**.

In the case of resin **16b**, the best conditions found for the cleavage of **16a** were used. Cleavage with $AlCl_3$ of a large amount of resin **16** (1 g) afforded only traces of **18** and the resin beads were found to break down under these conditions.[18] A reduction in the amount of $AlCl_3$ and the reaction time allowed the isolation of **18a** in good yield working on a 1 g scale of **16b**. The same results were obtained from the resins **16c** and **16d**, from which the bisarylpyrroles **18c** and **18d** were isolated. As observed previously, *N*-deprotection of pyrrole was concomitant during the cleavage. In addition, the application of these

Table 2. Solid-phase preparation of bisarylpyrrole **16a** and cleavage conditions



Entry	Coupling conditions[a]						Cleav. cond.[b]	Yield[c] (%)	
	Reag. A (equiv.)	Reag. B (equiv.)	Cat., Lig. (%)	Solvent	Temperature	Time (h)		**14**[d]	**18**[e]
1	*n*BuLi (10), $ZnCl_2$ (16)	 (10)	Pd_2dba_3, $AsPh_3$ (15, 40)	THF[f]	37 °C	21	$ZnBr_2$ AcBr		
2	*n*BuLi (10), $ZnCl_2$ (16)	 (10)	$PdCl_2(PPh_3)_2$, PPh_3 (25, 20)	THF[f]	rt	21	$ZnBr_2$ AcBr	Crude material difficult to manipulate	
3	*n*BuLi (10), $ZnCl_2$ (16)	 (10)	$Pd(PPh_3)_4$ (15)	THF[f]	rt	21	*$ZnBr_2$* AcBr		
4	—	 (4)	$Pd(PPh_3)_4$, 2 N Na_2CO_3 (10, 5)	THF	Reflux	19	$ZnBr_2$ AcBr	**a** (30) **b** (16)	**e** (20)
5	—	 (4)	$Pd(PPh_3)_4$, 2 N Na_2CO_3 (10, 5)	DME	Reflux	19	$ZnBr_2$ AcBr	**b** (28)	**e** (15) **f** (16)
6	—	 (4)	$Pd(PPh_3)_4$, 2 N Na_2CO_3 (10, 5)	Dioxane	Reflux	19	*$ZNBR_2$* AcBr	**a** (13) **b** (18)	**e** (22) **f** (10)
7	—	 (4)	$Pd(PPh_3)_4$, 2 N Na_2CO_3 (10, 5)	DME	Reflux	19	$AlCl_3$	**c** (46)	**a** (27)
8	—	 (4)	$Pd(PPh_3)_4$, 2 N Na_2CO_3 (10, 5)	Dioxane	Reflux	19	$AlCl_3$	**c** (32)	**a** (34)
9	—	 (4)	$Pd(PPh_3)_4$, 2 N Na_2CO_3 (10, 10)	Dioxane	Reflux	19	$AlCl_3$	**c** (15)	**a** (37)
10	—	 (10)	$Pd(PPh_3)_4$, 2 N Na_2CO_3 (20, 10)	Dioxane	Reflux	21	$AlCl_3$	(0)	**a** (84)

[a] 30–60 mg of **10** were used.
[b] Dry THF was used.
[c] All cleavage reactions were carried out in dry DCM at rt.
[d] Calculated from the HPLC of the crude cleavage product.
[e] tr **14a** 9.52 min, MS (380, 378); tr **14b** 7.90 min, MS (338, 336); tr **14c** 7.82 min, MS (298, 296).
[f] tr **18a** 8.74 min, MS (325); tr **18e** 10.12 min, MS (408); **18f** 8.82 min, MS (366).

cleavage conditions to resin **16b** also led to deprotection of the *O*-isopropoxy group to give lamellarin Q (**1**). Other Lewis acids, such as $SnCl_4$, reacted with **16b** to produce cleavage from the resin and *N*-deprotection but not the desired *O*-isopropoxy deprotection. This is a new example of how the choice of the appropriate cleavage reaction conditions allows different substituted derivatives to be obtained.[19]

N-Deprotection of **16a** and **16b** by treatment with NH_4F in DCM produced the anchored bisarylpyrroles **19a** and **19b**, respectively. The absence in the GP ^{13}C NMR of

characteristic signals for isopropylsilyl groups provided evidence that *N*-desilylation had occurred.

N-Alkylation of **19a** with *p*-methoxybromoacetylbenzene (**17a**) was investigated under different experimental conditions.[20] The use of excess NaH or LDA as a base[21] in dry THF under reflux gave, after cleavage with $AlCl_3$, moderate yields of the *N*-alkyl derivative **21a**.[22] Similar *N*-alkylation results were obtained starting with the *O*-isopropoxy-protected derivatives **19b** and **17a**.[23] Cleavage of the alkylation product **20b** with $AlCl_3$ afforded mixtures of **1** and **2**, as determined by HPLC and HPLC-MS.[22] The dimethylacetal of *p*-methoxybromoacetylbenzene[24] was prepared to avoid the possibility of enolate formation under the basic alkylation conditions but the N-alkylation process was not improved by this change.

Finally, other alkylating agents—MeI and the tosylate of 2-(2-bromophenyl)ethanol[25]—afforded resins **20d** and **20e**[26] which, after cleavage with $AlCl_3$ and purification by HPLC, produced *N*-methyllamellarin Q (**21c**) in moderate yield. However, **21d** proved impossible to isolate by HPLC.

An efficient solid-phase strategy has been devised for the preparation of the pyrrole-containing alkaloids, lamellarins Q and O. The process involves the incorporation of the appropriately substituted pyrrole ring onto a *p*-alkoxy iodophenyl resin through a Negishi cross-coupling reaction. This step is followed by a Suzuki cross-coupling reaction to introduce the second substituted phenyl ring and *N*-alkylation of the pyrrole. Final cleavage was achieved using a Lewis acid to give the desired product. Diversity can be introduced in each step of the synthetic process as well as during the final cleavage by using the appropriate Lewis acid conditions. GP ^{13}C NMR has proven to be a good method to follow the different steps in the process.

3. Experimental

3.1. General

Melting points were determined in capillary tubes and are uncorrected. TLC was carried out on SiO_2 (silica Gel 60 F_{254}, Merck) and spots were located with UV light. Column chromatography was carried out on SiO_2 (silica Gel 60 SDS 0.035–0.070 mm). Organic extracts were dried over anhydrous Na_2SO_4 and solutions were evaporated under reduced pressure with a rotatory evaporator. IR spectra were obtained using a Thermo Nicolet Nexus spectrophotometer. NMR spectra were acquired with Varian Gemini-200 (200 MHz), Varian Gemini-300 (300 MHz), Mercury-400 (400 MHz) and Varian VXR-500 (500 MHz) spectrometers; data are given on the δ scale referenced to TMS. Mass spectra were measured in the electron impact (EI) mode with a Hewlett–Packard model 5989A spectrometer. High resolution mass spectra were performed on an Auto-Spec/VG by Unidad de Espectrometría de Masas de Santiago de Compostela. HPLC was carried out using a Waters 996 Photodiode Array Detector, a SYMMETRY C_{18} column (4.6×150 mm, 5 μm) and H_2O (0.045% TFA) and AcCN (0.036% TFA) as eluents. HPLC-MS was carried out on a Waters micromass ZQ using a SYMMETRY C_{18} column (3.9×150 mm, 5 μm), Waters 2487 Dual Absorbance Detector and H_2O (0.1% formic acid) and AcCN (0.07% formic acid) as eluents. Purification by HPLC was carried out with Waters 2487 Dual Absorbance Detector, Waters 600 Controller and Waters Fraction Collector II using a SYMMETRY C_{18} (30×100 mm, 5 μm) column and H_2O (0.1% TFA) and AcCN (0.05% TFA) as eluents. Solid-phase reactions were shaken in a Vibromatic SELECTA. Microwave reactions were carried out in a DISCOVER (CEM).

3.1.1. 3,4-Dibromo-1-(triisopropylsilyl)pyrrole-2-carboxylic acid 7b.[4b] *n*BuLi (1.36 ml, 2.17 mmol, 1.6 M) was added dropwise to a cooled (−78 °C) solution of 2,3,4-tribromo-1-trimethylsilanylpyrrole (1 g, 2.17 mmol) in dry THF (15 ml) under N_2 and the mixture was stirred for 15 min at that temperature. Dry CO_2 was bubbled through the mixture at that temperature and the cooling bath was removed. The reaction mixture was stirred at rt for 20 min. The solution was diluted with H_2O, acidified to pH 1 with 1 N HCl and extracted with EtOAc. The organic layer was dried and evaporated under vacuum. The crude product was purified by flash chromatography. Elution with hexane/DCM (8:2) gave **7b** as a white solid (425 mg, 46%). mp 142.3–143.3 °C (hexane/EtOAc). IR (KBr) ν 3272, 1653, 1460, 825; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.14 (d, J=7.7 Hz, 18H, CH_3), 1.41 [m, 1H, $CH(CH_3)_2$], 6.98 (d, J=3.3 Hz, 1H, H5), 9.65 (bs, 1H, CO_2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.3 [q, $(CH_3)_2$], 17.8 [d, $CH(CH_3)_2$], 102.7 (s, C4), 106.3 (s, C3), 121.8 (s, C2), 122.5 (d, C5), 159.4 (s, C=O); MS (EI) m/z 428 (2 $^{81}BrM^+$, 19), 426 ($^{81}BrM^+$, $^{79}BrM^+$, 34), 424 (2 $^{79}BrM^+$, 18), 384 (2 $^{81}BrM^+$, 54), 382 ($^{81}BrM^+$, $^{79}BrM^+$, 100), 380 (2 $^{79}BrM^+$, 50); HRMS (EI) m/z calculated for $C_{13}H_{24}NO_2Br_2Si$: 426.9956; found: 426.9940.

3.1.2. 3,4-Dibromo-1-triisopropylsilylpyrrole-2-carboxylate resin 9. Merrifield-OH resin (1 g, loading 0.68 mmol/g) was swelled in dry DMF and **7b** (289 mg, 1 equiv.) and DMAP (41 mg, 0.5 equiv.) in DMF (0.5 ml) and DIPCDI (106 μl, 1 equiv.) were added. The reaction mixture was stirred at rt for 3 h. The resin was washed with DMF and a second treatment with the same quantities of **7b**, DMAP and DIPCDI was carried out. The reaction mixture was stirred for 3 h at rt. Finally, the resin was washed with DMF, DCM, MeOH and Et_2O and dried under vacuum.

3.1.3. Methyl 3,4-dibromopyrrole-2-carboxylate 7c. Resin **9** (100 mg) was swelled in DCM (5 ml) and LiOH (32.5 mg, 20 equiv.) in MeOH (5 ml) was added. The reaction mixture was shaken at reflux temperature for 2 h. The resin was washed with DCM and the organic layer was evaporated. The crude product was diluted in H_2O, acidified to pH 2 with 1 N HCl and extracted with EtOAc. The organic layer was dried and evaporated under vacuum to give the title compound (3.5 mg of crude material). The spectroscopic data of the product are the same as described previously.[4b] IR (NaCl) ν 3359,31, 1728, 1368; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.91 (s, 3H, CO_2CH_3), 7.00 (d, J=2.8 Hz, 1H, H5); MS (EI) m/z 285 (2 $^{81}BrM^+$, 31), 283 ($^{81}BrM^+$, $^{79}BrM^+$, 63), 281 (2 $^{79}BrM^+$, 33), 253 (2 $^{81}BrM^+$, 50), 251 ($^{81}BrM^+$, $^{79}BrM^+$, 100), 249 (2 $^{79}BrM^+$, 51).

3.1.4. 4-(4-Bromo-2-methoxycarbonyl-1-triisopropylsilylpyrrol-3-yl)phenoxy resin 10. *n*-BuLi (8.13 ml, 10 equiv., 1.6 M) was added dropwise to a cooled (−78 °C) solution of methyl 3,4-dibromo-1-trimethylsilanylpyrrole-2-carboxylate (5.71 g, 10 equiv.) in dry THF (35 ml) under N_2 and the mixture was stirred for 15 min at −78 °C. A solution of $ZnCl_2$ (2.84 g, 16 equiv.) in dry THF (8 ml) was added and the reaction mixture was stirred for 5 min at −78 °C and 25 min at rt. The reaction mixture was transferred using N_2 to a mixture of the swelled resin **11** (2.82 g) with dry THF (15 ml) and $Pd(PPh_3)_4$ (225 mg, 0.15 equiv.). The reaction mixture was shaken for 24 h at rt and, after this time, was washed with THF, DCM, MeOH, Et_2O (3×10 ml, each) and finally was dried in a vacuum oven at 40 °C. IR (KBr) ν 1697 (C═O), 1600, 741, 700; ^{13}C MAS NMR ($CDCl_3$, 125 MHz) δ 13.4 [$CH(CH_3)_2$], 18.3 ($CHCH_3$), 50.9 (CO_2CH_3).

3.1.5. 4-Iodophenoxy-resin 11.[27] Merrifield-Cl resin (4 g, loading 0.5 mmol/g) was swelled in dry DMF (60 ml) for 30 min and 4-iodophenol (2.20 g, 5 equiv.) and NaOMe (22.3 ml, 5 equiv., 4.4 M) in dry DMF were added. The reaction mixture was warmed at 80 °C and shaken for 24 h. The resin was washed with DCM, DMF/DCM (1:1), DMF, DCM, MeOH, Et_2O (3×3 ml, each) and was dried under vacuum. The resin was swelled in dry DMF (60 ml) for 15 min, NaOMe (1.2 ml, 4 equiv., 4.4 M) was added and the mixture was shaken for 3 h at 80 °C. The resin was washed with DMF, DCM, MeOH and Et_2O (3×10 ml) and dried under vacuum. IR (KBr) ν 1943, 1872, 1803, 1599, 743, 698.

3.2. General procedure for cleavage with $ZnBr_2$/AcBr

$ZnBr_2$ (3.5 equiv.) and AcBr (40 equiv.) were added to the swelled resin in DCM under N_2 and the reaction mixture was shaken for 24 h at rt. The resin was filtered off and washed with DCM. The organic solution was washed with 5% aq. $NaHCO_3$, 5% aq. HCl, saturated NaCl, dried and evaporated.

3.2.1. 4-Iodophenol acetate 13a. The general procedure for cleavage with $ZnBr_2$/AcBr on resin **11** (40 mg) gave **13a** (2.5 mg, 50%). ^1H NMR ($CDCl_3$, 200 MHz)[28] δ 2.29 (s, 3H, CH_3), 6.86 (d, J=8.8 Hz, 2H, H2, H6), 7.68 (d, J=8.8 Hz, 2H, H3, H5).

3.2.2. Methyl 3-(4-acetoxyphenyl)-5-acetyl-4-bromopyrrole-2-carboxylate 14a. The general procedure for cleavage with $ZnBr_2$/AcBr on resin **10** (110 mg) gave **14a** (17 mg). HPLC (35–50% ACN in 15 min; tr 12.28 min, 83% purity). ^1H NMR ($CDCl_3$, 200 MHz) δ 2.33 (s, 3H, $COCH_3$), 2.72 (s, 3H, $OCOCH_3$), 3.75 (s, 3H, CO_2CH_3), 7.17 (d, J=8.6 Hz, 2H, H3′, H5′), 7.35 (d, J=8.6 Hz, 2H, H2′, H6′); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 17.7 (q, CH_3), 21.3 (q, CH_3), 52.2 (q, CH_3), 104.9 (s), 120.8 (d), 122.2 (s), 129.4 (s), 130.6 (s), 130.9 (s), 131.6 (d), 150.4 (s), 165.1 (s), 169.2 (s), 188.2 (s). MS(EI) m/z 381 ($^{81}BrM^+$, 11), 379 ($^{79}BrM^+$, 11), 337 (74), 339 (72), 307 (74), 305 (74), 292 (40), 290 (40). HRMS (EI) m/z calculated for $C_{16}H_{14}NO_5Br$: 379.0055; found: 379.0066.

3.3. General procedure for the preparation of 16

Resin **10** was swelled with dioxane for 15 min. 2 M aq. Na_2CO_3 (10 equiv.), boronic acid **15** (10 equiv.) and $Pd(PPh_3)_4$ (0.2 equiv.) were added and the reaction mixture was shaken under reflux between 21 and 48 h. Resin **16** was washed with dioxane, DCM, MeOH, Et_2O (3×4 ml, each) and was dried under vacuum.

3.3.1. 4-[2-Methoxycarbonyl-4-(4-methoxyphenyl)-1-triisopropylsilylpyrrol-3-yl]phenoxy resin 16a. The general diaryl synthesis procedure, with resin **10** (1 g) and 4-methoxyphenylboronic acid, gave resin **16a** after a reaction time of 19 h. IR (KBr) ν 1691 (C═O), 1600, 1450, 746. ^{13}C NMR ($CDCl_3$, Gel Phase, 75 MHz) δ 13.6 [$CH(CH_3)_2$], 18.6 [$CH(CH_3)_2$], 53.4 (CO_2CH_3), 67.0 (OCH_3).

3.3.2. 4-[2-Methoxycarbonyl-4-(4-isopropoxyphenyl)-1-triisopropylsilylpyrrol-3-yl]-phenoxy resin 16b. The general diaryl synthesis procedure, with resin **10** (1 g) and 4-isopropoxyphenylboronic acid,[16] gave resin **16b** after a reaction time of 48 h. IR (KBr) ν 1695 (C═O), 1600, 744. ^{13}C NMR ($CDCl_3$, Gel Phase, 75 MHz) δ 13.6 [$CH(CH_3)_2$], 18.6 [$CH(CH_3)_2$], 22.1 [$OCH(CH_3)_2$], 53.4 (CO_2CH_3), 69.6 [$OCH(CH_3)_2$].

3.3.3. 4-[2-Methoxycarbonyl-4-(3,4-dimethoxyphenyl)-1-triisopropylsilylpyrrol-3-yl]phenoxy resin 16c. The general diaryl synthesis procedure, with resin **10** (1 g) and 3,4-dimethoxyphenylboronic acid, gave resin **16c** after a reaction time of 48 h. IR (KBr) ν 1694 (C═O), 1600, 1492, 744. ^{13}C NMR ($CDCl_3$, Gel Phase, 75 MHz) δ 13.6 [$CH(CH_3)_2$], 18.6 [$CH(CH_3)_2$], 55.2 (−OCH_3), 55.7 (−OCH_3).

3.3.4. 4-[2-Methoxycarbonyl-4-(2-naphthyl)-1-triisopropylsilylpyrrol-3-yl]-phenoxy resin 16d. The general diaryl synthesis procedure, with resin **10** (1 g) and 2-naphthaleneboronic acid, gave resin **16d** after a reaction time of 48 h. IR (KBr) ν 1694 (C═O), 1600. ^{13}C NMR ($CDCl_3$, Gel Phase, 75 MHz) δ 13.6 [$CH(CH_3)_2$], 18.6 [$CH(CH_3)_2$].

3.4. General procedure for cleavage with $AlCl_3$

$AlCl_3$ (3 equiv.) was added to the swelled resin **16** in dry DCM under N_2. The reaction mixture was shaken for 3 h at rt. The resin was washed with DCM. The organic layer was washed with 10% aq. HCl, dried and evaporated to give **18**. The crude material was analysed by HPLC-MS.

3.4.1. Methyl 3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)pyrrole-2-carboxylate 18a. Resin **16a** (600 mg) gave **18a** by following the general procedure for cleavage with $AlCl_3$ (15 equiv.). The crude material was analysed by HPLC-MS (gradient 30–70% ACN in 15 min), **18a** (tr 5.67 min, MS 324, M+1). Purification by HPLC (gradient 20–65% AcCN in 40 min) gave **18a** (1 mg, 2%) as a pale-yellow solid. ^1H NMR ($CDCl_3$, 500 MHz) δ 3.73 (s, 3H, CO_2CH_3), 3.76 (s, 3H, OCH_3), 6.76 (bt, J=8.7 Hz, 4H, H3′, H5′, H3″, H5″), 7.00–7.06 (m, 3H, H2′, H6′, H5), 7.14 (d, J=8.5 Hz, 2H, H2″, H6″), 9.12 (bs, NH/OH). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 51.4 (s, CO_2CH_3), 55.2 (s, OCH_3), 113.8 (d, C3″, C5″), 114.8 (d, C3′, C5′), 129.6 (d, C2′, C6′), 132.2 (d, C2″, C6″). MS (EI) m/z 324 (M+1, 12), 323 (M^+, 9), 292 (18). HRMS (EI) m/z calculated for $C_{19}H_{17}NO_4$: 323.1157; found: 323.1157.

3.4.2. Methyl 3,4-bis(4-hydroxyphenyl)pyrrole-2-carboxylate 1: lamellarin Q. Resin **16b** (500 mg), gave **1** by following the general procedure for cleavage with $AlCl_3$. The crude product was analysed by HPLC-MS (gradient 20–40% ACN in 15 min) and **1** (tr 6.92 min, MS 310, M+1). Purification by HPLC (gradient 20–40% ACN in 20 min) gave **1** (5 mg, 13%) as a pale-yellow gum. ^{1}H NMR (Acetone-d^6, 400 MHz) δ 3.64 (s, 3H, CO_2CH_3), 6.66 (d, J=8.4 Hz, 2H, H3′, H5′), 6.76 (d, J=8.4 Hz, 2H, H3″, H5″), 6.95 (d, J=8.4 Hz, 2H, H2″, H6″), 7.05 (d, J=8.4 Hz, 2H, H2″, H6″), 7.13 (bs, 1H, H5), 8.25 (bs, 2H, OH), 10.91 (bs, 2H, NH). ^{13}C NMR (Acetone-d^6, 100 MHz) δ 50.3 (q, CO_2CH_3), 114.4 (d, C3″, C5″), 115.0 (d, C3′, C5′), 120.5 (d, C5), 120.7 (s, C2), 126.1 (s, C4), 126.2 (s, C3), 126.7 (s, C1″), 129.0 (s, C1), 129.5 (d, C2′, C6′), 132.1 (d, C2″, C6″), 155.8 (s, C4″), 156.3 (s, C4″), 161.2 (s, C=O). MS(EI) *m/z* 310 (M+1, 25), 309 (M$^+$, 22), 278 (100); HRMS (EI) *m/z* calculated for $C_{18}H_{15}NO_4$: 309.1001; found: 309.1012.

3.4.3. Methyl 3-(4-hydroxyphenyl)-4-(4-isopropoxyphenyl)pyrrole-2-carboxylate 18b. Resin **16b** (100 mg) was swelled in DCM for 10 min, $SnCl_4$ (10 equiv.) was added and the mixture was shaken at rt for 12 h. The resin was washed with DCM and the organic layer was washed with 10% aq. HCl, dried and evaporated. The crude material was analysed by HPLC-MS (gradient 20–40% ACN in 15 min) and **18b** (tr 10.09 min, MS 352) was obtained. Purification by HPLC (gradient 50–70% ACN in 20 min) gave **18b** (1 mg, 11%) as a white solid. ^{1}H NMR (Acetone-d^6, 500 MHz) δ 1.27 [d, J=6 Hz, 6H, $(CH_3)_2$], 3.65 (s, 3H, CO_2CH_3), 4.55 [h, J=6 Hz, 1H, $CH(CH_3)_2$], 6.73 (d, J=8.7 Hz, 2H, H3′, H5′), 6.76 (d, J=8.7 Hz, 2H, H3″, H5″), 7.03 (d, J=8.7 Hz, 2H, H2′, H6′), 7.06 (d, J=8.7 Hz, 2H, H2″, H6″), 7.17 (d, J=3 Hz, 1H, H5). ^{13}C NMR (Acetone-d^6, 100 MHz) δ 22.1 [q, $(CH_3)_2$], 50.3 (q, CO_2CH_3), 69.4 [d, $CH(CH_3)_2$], 114.5 (d, C3″, C5″), 115.5 (d, C3′, C5′), 120.8 (d, C5), 125.8 (s, C1″), 125.9 (s, C1′),128.8 (s, C3), 129.4 (d, C2′, C6′), 131.8 (s, C4), 132.1 (d, C2″, C6″), 156.1 (s, C4′), 156.3 (s, C4″), 162.6 (s, C=O). MS (EI) *m/z* 352 (M+1, 15), 351 (M$^+$, 11), 320 (38). HRMS (EI) *m/z* calculated for $C_{21}H_{21}NO_4$: 351.1470; found: 351.1465.

3.4.4. Methyl 3-(4-hydroxyphenyl)-4-(3,4-dimethoxyphenyl)pyrrole-2-carboxylate 18c. Resin **16c** (820 mg) gave **18c** by following the general procedure for cleavage with $AlCl_3$. The crude material was analysed by HPLC-MS (gradient 20–40% ACN in 15 min), **18c** (tr 10.09 min, MS 354, M+1). Purification by HPLC (gradient 25–40% ACN in 30 min) gave **18c** (10 mg, 14%) as a white solid. ^{1}H NMR (Acetone-d^6, 500 MHz) δ 2.98 (bs, 1H, OH/NH), 3.52 (s, 3H, C4″–OCH_3), 3.65 (s, 3H, CO_2CH_3), 3.75 (s, 3H, C3″–OCH_3), 6.63 (d, J=2 Hz, 1H, H2″), 6.77–6.81 (m, 3H, H5′, H3′, H6″), 7.07 (d, J=8.5 Hz, 2H, H6′, H2′), 7.23 (d, J=3.5 Hz, 1H, H5), 10.96 (bs, 1H, NH/OH). ^{13}C NMR (Acetone-d^6, 100 MHz) δ 50.3 (q, CO_2CH_3), 54.9 (q, OCH_3), 55.4 (q, OCH_3), 112.0 (d, C6″), 112.6 (d, C2″), 114.5 (d, C5′, C3′), 119.4 (s, C2), 120.2 (d, C5″), 120.6 (d, C5), 120.8 (s, C4), 126.4 (s, C1″), 128.3 (s, C3), 129.1 (s, C1′), 132.1 (s, C6′, C2′), 147.9 (s, C3″), 149.1 (s, C4″), 156.4 (s, C4′), 161.2 (s, C=O). MS (EI) *m/z* 354 (M+1, 39), 353 (M$^+$, 31), 322 (100). HRMS (EI) *m/z* calculated for $C_{20}H_{19}NO_5$: 353.1263; found: 353.1261.

3.4.5. Methyl 3-(4-hydroxyphenyl)-4-(2-naphthyl)-pyrrole-2-carboxylate 18d. Resin **16d** (700 mg) gave **18d** by following the general procedure for cleavage with $AlCl_3$. The crude material was analysed by HPLC-MS (gradient 30–70% ACN in 15 min), **18d** (tr 8.6 min, MS 344, M+1). Purification by HPLC (gradient 35–65% AcCN in 30 min) gave **18d** (1 mg, 2%) as a white solid. ^{1}H NMR (Acetone-d^6, 500 MHz) δ 3.67 (s, 3H, CO_2CH_3), 6.76 (d, J=9 Hz, 2H, H3′, H5′), 7.10 (d, J=9 Hz, 2H, H2′, H6′), 7.24 (dd, J=8, 1.5 Hz, 1H, H3″), 7.4 (m, 3H, H5, H5″, H8″), 7.67 (m, 3H, H1″, H6″, H7″), 7.79 (m, 1H, H4″). MS (EI) *m/z* 344 (M+1, 19), 343 (M$^+$, 59). HRMS (EI) *m/z* calculated for $C_{22}H_{17}NO_3$: 343.1208; found: 343.1193.

3.5. General procedure for *N*-desilylation

Resin **16** was swelled in DCM for 10 min and a solution of NH_4F (7 equiv.) in MeOH was added. The mixture was shaken under reflux for 6 h. The resin was washed with DCM and MeOH (5×4 ml, each) and dried.

3.5.1. 4-[2-Methoxycarbonyl-4-(4-methoxyphenyl)-pyrrol-3-yl]phenoxy resin 19a. Resin **16a** (2 g) gave resin **19a** by following the general procedure for desilylation. IR (KBr) ν 3414, 3286, 1690, 1600, 1451, 756, 695. ^{13}C NMR ($CDCl_3$, Gel Phase, 75 MHz) δ 53.4 (CO_2CH_3), 69.8 (OCH_3).

3.5.2. 4-[2-Methoxycarbonyl-4-(4-isopropoxyphenyl)-pyrrol-3-yl phenoxy resin 19b. Resin **16b** (370 mg) gave resin **19b** by following the general procedure for desilylation. IR (KBr) ν 3430, 3287, 1690 (C=O), 1600, 755. ^{13}C NMR ($CDCl_3$, Gel Phase, 75 MHz) δ 22.0 [$OCH(CH_3)_2$], 53.3 (CO_2CH_3), 69.6 [$OCH(CH_3)_2$].

3.5.3. 4-{2-Methoxycarbonyl-4-(4-methoxyphenyl)-1-[2-(4-methoxyphenyl)-2-oxoethyl]pyrrol-3-yl}-phenoxy-resin 20a. LDA (150 μl, 2 equiv.) was added dropwise to swelled resin **19a** (370 mg) in dry THF (10 ml) under N_2 at −78 °C. The resin was shaken for 1 h at this temperature. 2-Bromo-4′-methoxyacetophenone (172 mg, 5 equiv.) was added. The cooling bath was removed and the crude mixture was shaken in a vibromatic at 86 °C for 24 h. After this time the resin was washed with THF, DCM, MeOH and Et_2O (3×5 ml, each) and dried under vacuum. IR (KBr) ν 3400, 2920, 1691, 1600. ^{13}C NMR ($CDCl_3$, 75 MHz) δ 55.2 (CO_2CH_3), 69.9 (OCH_3).

3.5.4. 4-{2-Methoxycarbonyl-4-(4-isopropoxyphenyl)-1-[2-(4-methoxyphenyl)-2-oxoethyl]pyrrol-3-yl}phenoxy-resin 20b. Resin **19b** (500 mg) was swelled in a solution of 18-crown-6 in DMF (2.5 M, 25 ml) for 10 min. K_2CO_3 (6 equiv.) and 2-bromo-4′-methoxyacetophenone (6 equiv.) were added. The reaction mixture was heated in a microwave oven at 100 °C and 30–40 W during 2 min. The resin was washed with DMF, DMF/H_2O (1:1), DCM, MeOH and Et_2O (3×5 ml, each) and dried under vacuum. **20b** was obtained. IR (KBr) ν 3417, 1724(C=O), 1690 (C=O), 1600. ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.1 [$OCH(CH_3)_2$], 59.8 (CO_2CH_3), 69.6 [$OCH(CH_3)_2$].

3.5.5. 4-[2-Methoxycarbonyl-4-(4-isopropoxyphenyl)-1-methylpyrrol-3-yl]phenoxy resin 20d. Resin **19b**

(300 mg) was swelled in a solution of 18-crown-6 in DMF (2.5 M) for 10 min and K_2CO_3 (15 equiv.) and MeI (6 equiv.) were added. The reaction mixture was shaken at rt for 24 h. The resin was washed with DMF, DMF/H_2O (1:1), DCM, MeOH and Et_2O (3×5 ml, each) and dried under vacuum. **20d** was obtained. IR (KBr) ν 1693, 1600, 1492, 743. ^{13}C NMR ($CDCl_3$, Gel Phase, 75 MHz) δ 22.1 [OCH(CH_3)$_2$], 69.6 [OCH(CH$_3$)$_2$].

3.5.6. 4-{2-Methoxycarbonyl-4-(4-isopropoxyphenyl)-1-[2-(2-bromophenethyl)]pyrrol-3-yl}-phenoxy-resin 20e. Resin **19b** (500 mg) was swelled in a solution of 18-crown-6 in DMF (2.5 M, 25 ml) for 10 min and K_2CO_3 (4 equiv.) and 2-bromophenethyl tosylate (4 equiv.) were added. The reaction mixture was shaken at 80 °C for 24 h. After this time, the resin was washed with DMF, DMF/H_2O (1:1), DCM, MeOH and Et_2O (3×5 ml, each) and dried under vacuum. **20e** was obtained. IR (KBr) ν 1642, 610. ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.1 [OCH(CH_3)$_2$], 69.6 [OCH(CH$_3$)$_2$].

3.5.7. Methyl 3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-1-[2-(4-methoxyphenyl)-2-oxoethyl] pyrrole-2-carboxylate 21a. Resin **20a** (300 mg) gave **21a** by following the general procedure for cleavage with $AlCl_3$ (15 equiv.) for 6 h. The crude product was analysed by HPLC-MS (gradient 35–50% ACN in 15 min): **21a** [tr 12.89 min, MS (472, M+1)]. Purification by HPLC (gradient 30–60% ACN in 30 min) gave **21a** (1.0 mg, 2%) as a yellow solid.^1H NMR ($CDCl_3$, 600 MHz) δ 3.45 (s, 3H, CO_2Me), 3.81 (s, 3H, OMe), 3.86 (s, 3H, OMe), 5.72 (s, 2H, CH_2), 6.89 (d, 2H, J=9 Hz, H3″, H5″), 7.00 (s, 1H, H5), 7.05 (d, 2H, J=8.4 Hz, H3‴, H5‴), 7.08 (d, 2H, J=7.6 Hz, H3′, H5′), 7.16 (d, 2H, J=7.6 Hz, H2′, H6′), 7.44 (d, 2H, J=8.4 Hz, H2″, H6″), 8.03 (d, 2H, J=9 Hz, H2‴, H6‴).

3.5.8. Methyl 3,4-bis(4-hydroxyphenyl)-1-methylpyrrole-2-carboxylate 21c. Resin **20d** (500 mg) gave **21c** by following the general procedure for cleavage with $AlCl_3$. The crude product was analysed by HPLC-MS (gradient 30–70% ACN in 15 min): **21c** (tr 5.67 min, MS 324, M+1). Purification by HPLC (gradient 20–70% ACN in 50 min) gave **21c** (1.7 mg, 6%) as a brown solid. ^1H NMR (Acetone-d^6, 500 MHz) δ 2.92 (bs, OH), 3.53 (s, 3H, CO_2CH_3), 3.95 (s, 3H, N–CH_3), 6.64 (d, J=8.5 Hz, 2H, H3′, H5′), 6.75 (d, J=8.5 Hz, 2H, H3″, H5″), 6.91 (d, J=8.5 Hz, 2H, H2′, H6′), 6.97 (d, J=8.5 Hz, 2H, H2″, H6″), 7.10 (s, 1H, H5). ^{13}C NMR (Acetone-d^6, 100 MHz) δ 36.9 (q, CH_3), 50.0 (q, CO_2CH_3), 112.4 (d, C3″, C5″), 115.0 (d, C3′, C5′), 120.6 (s, C2), 124.1 (s, C4), 126.6 (s, C1′), 127.0 (d, C5), 127.4 (s, C1″), 129.4 (d, C2′, C5′), 130.6 (s, C3), 131.9 (d, C2″, C5″), 155.8 (s, C4′), 156.3 (s, C4″), 162.6 (s, C=O). MS(EI) m/z 324 (M+1, 14), 323 (M$^+$, 23), 292 (58). HRMS (EI) m/z calculated for $C_{19}H_{17}NO_4$: 323.1158; found 323.1149.

3.5.9. Methyl 3,4-bis(4-hydroxyphenyl)-1-[2-(2-bromophenyl)ethyl]pyrrole-2-carboxylate 21d. Resin **20c** (500 mg) gave **21d** by following the general procedure for cleavage with $AlCl_3$. The crude product was analysed by HPLC-MS (gradient 30–70% ACN in 15 min): **21d** [tr 12.64 min, MS (492, M+1)].

3.5.10. Methyl 3,4-bis-(4-hydroxyphenyl)-1-[2-(4-methoxyphenyl)-2-oxoethyl]pyrrole-2-carboxylate 2: lamellarin O. Resin **20b** (500 mg) gave **2** by following the general procedure for cleavage with $AlCl_3$. The crude product was analysed by HPLC-MS (gradient 40–80% ACN in 15 min): **2** [tr 11.94 min, MS 458 (M+1)].

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11. Formation of **10** was investigated by ^{13}C MAS NMR and corroborated by characterisation of the diacetyl derivative **14a** obtained by the cleavage reaction of **10** with $ZnBr_2$ and AcBr.
12. The crude cleavage material was analysed by HPLC and HPLC-MS.
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14. Commercial 3-methoxyphenylboronic acid was used to find the best reaction conditions to be applied to the synthesis of lamellarins and derivatives.
15. Mixtures of **18e** and **18f**, as well as of **14a** and **14b**, in different proportions were produced during the cleavage with $ZnBr_2$ in AcBr due to electrophilic acetylation on the pyrrole. Compound **18f** was not isolated but detected by HPLC-MS and its structure postulated on the basis of the ^1H NMR spectrum of the crude mixture by the singlet at 7.29 ppm due to the pyrrole proton 5.
16. GP ^{13}C NMR of bisarylpyrroles **16b**-**16d** were used to corroborate the introduction of the new aromatic ring.
17. The 4-isoproxoxyphenylboronic acid **15b** was not commercial when we started this project. It was prepared from the *p*-iodophenol by protection with 2-iodopropane followed by boronic acid formation via halogen–metal interchange using *n*BuLi followed by reaction with trimethylboronate as described in Chaumeil, H.; Signorella, S.; Le Drian, C. *Tetrahedron* **2000**, *56*, 9655.
18. The stability of chloro-Merrifield resin under the same reaction conditions, i.e. large excess of $AlCl_3$ in DCM at rt during 3 h, was tested and the result was the total breakdown of the resin beads. Less harsh conditions involving an excess of 3 equiv. of $AlCl_3$ over the resin during 3 h allowed total recovery of the resin beads.
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21. 15 equiv. of NaH or 2 equiv. of LDA in dry THF at reflux for 24 h afforded, after cleavage, mixtures of **18a** and **21a** in which the alkylation product was obtained in 25% (NaH) and 30% (LDA) yield (HPLC-MS). Increasing the amount of LDA or the use of other bases did not improve the yield of **21a**.
22. The compound was not isolated after trying different purification procedures on the crude cleavage material by semipreparative HPLC. The loss of material, probably by decomposition in the column.
23. The best results were obtained using an excess of K_2CO_3 and 18-crown-6 as base in DMF in a microwave at 100 °C and 30–40 W during 2 min.
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Gaining diversity in solid-phase synthesis by modulation of cleavage conditions from hydroxymethyl-based supports. Application to lamellarin synthesis

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Abstract—The application of a number of Lewis acids as a cleavage/deprotection method in the solid-phase synthesis of organic molecules can render several analogues, which, after purification, can be submitted for biological evaluation.

1. Introduction

Although combinatorial chemistry has evolved greatly since its early days in peptide chemistry, it is undoubtedly an essential tool in modern programs of medicinal chemistry.[1] Combinatorial chemistry is currently being redirected towards the rapid and rational preparation of small and medium sized libraries of compounds based on a determined scaffold.[2]

Library preparation can be carried out in solid-phase[3] or in solution, by mainly taking advantage of the solid-phase mode through the use of supported reagents and/or supported purification probes.[4] Library diversity is normally introduced during the incorporation of the building blocks. However, when the library is prepared in solid-phase, diversity can also be introduced during cleavage. Thus, there are resins specially designed for this purpose.[5] These resins usually release the final compounds by means of a nucleophile. The oxime resin developed by Kaiser and DeGrado[6] and the 'safety-catch' resins developed by Kenner[7] and Ellman,[8] and the aryl hydrazine linker of Lowe[9] are examples of it.[10] Another new concept introduced by combinatorial chemistry is that any side-product is valuable because after purification it can be submitted for biological screening.[11]

Here we describe an example of how the synthesis of the distinct analogues of a natural product can be obtained by using different cleavage conditions, and even by modulating these conditions.

For this study, lamellarins were chosen as substrates. These hexacyclic alkaloids were first isolated from *Lamellarina* sp in 1985 by Faulklner et al.[12] Some lamellarins exhibit a wide array of interesting and significant biological activities, which include cell division inhibition, cytotoxicity, HIV-1 integrasa inhibition and immunomodulation.[13] While the first synthesis of lamellarins was carried out in 1997, our group has recently reported the first solid-phase synthesis of these hexacyclic alkaloids (Fig. 1).[14]

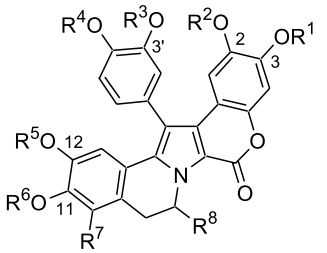

Figure 1. General formula of lamellarins.

2. Results and discussion

Some lamellarins have phenol functions at C3 and C3′ (R^1, R^3=H), therefore a plausible way to start solid-phase synthesis is by anchoring the phenol at C3′ to a solid support. Merrifield and Wang resins are convenient for this process because they shown a good mechanical and

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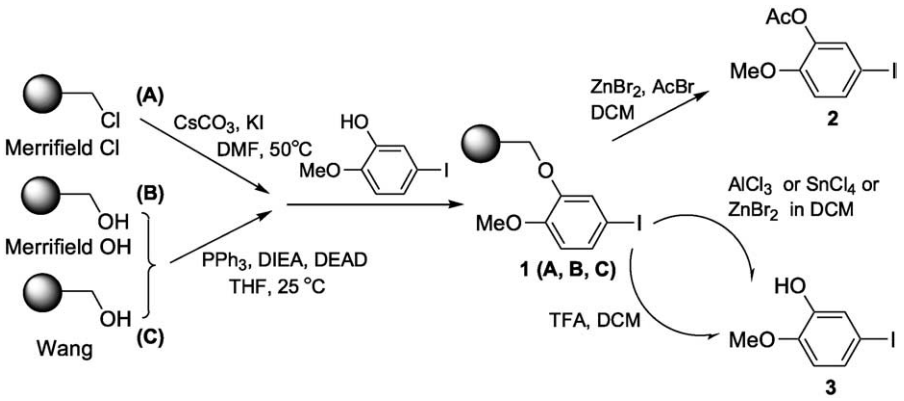

Figure 2. Incorporation and cleavage of the first building block.

chemical stability, highest loading and the lowest cost, which make these resins the most suitable solid supports for a multi-step solid-phase synthesis.[5] These resins, which are widely used in peptide synthesis for the preparation of acid peptides, release the final compounds after an acid treatment. While TFA (for the Wang resin), which is the method of choice for peptide cleavage, is a convenient reagent for combinatorial chemistry, its counterparts for Merrifield resin, anhydrous HF or TFMSA, do not appear to be appropriate. During recent years several methods based on the use of Lewis acids have been used to cleave small molecules from this type of resins.[15] Thus, here we have checked some of these methods in the synthesis of lamellarins to obtain different analogues. Resins were cleaved with a Lewis acid such as $AlCl_3$,[16] $SnCl_4$,[17] $ZnBr_2$[18] or with carboxylic acids such as TFA in DCM.

Three different polystyrene resins, chloro and hydroxy Merrifield and Wang resins were tested. 2-Methoxy-5-iodophenol was incorporated to Merrifield-Cl resin by the cesium salt method ($CsCO_3$, KI, in DMF at 50 °C for 24 h, the process was repeated once), and to the hydroxy resins via Mitsunobu conditions (PPh_3, DIEA, DEAD in THF at 25 °C for 3 h). Furthermore, Merrifield-Cl was also rejected because the incorporation of the phenol required more drastic conditions (double process at 50 °C for 24 h) and because the reaction cannot be followed by any colorimetric test or FT-IR.[19]

Initially, 2-methoxy-5-iodophenol was recovered with good yield and purity in all the cleavage cases, but in the $SnCl_4$ cleavage the purity of the compound was not acceptable. Thus, we discarded this reagent. When the $ZnBr_2$ cleavage is carried out into the presence of acetyl bromide the corresponding acetyl derivative is obtained with good yield and purity. Thus seems, the possibility of using another acyl bromide to increase the diversity (Fig. 2).

Lamellarin U (R^2, R^3=H) was taken as a model and its structure was built up in the two hydroxymethyl resins. The hydroxyl group of the OH in position 3 was protected as isopropyl ether, which can be cleaved by acids,[20] if it is

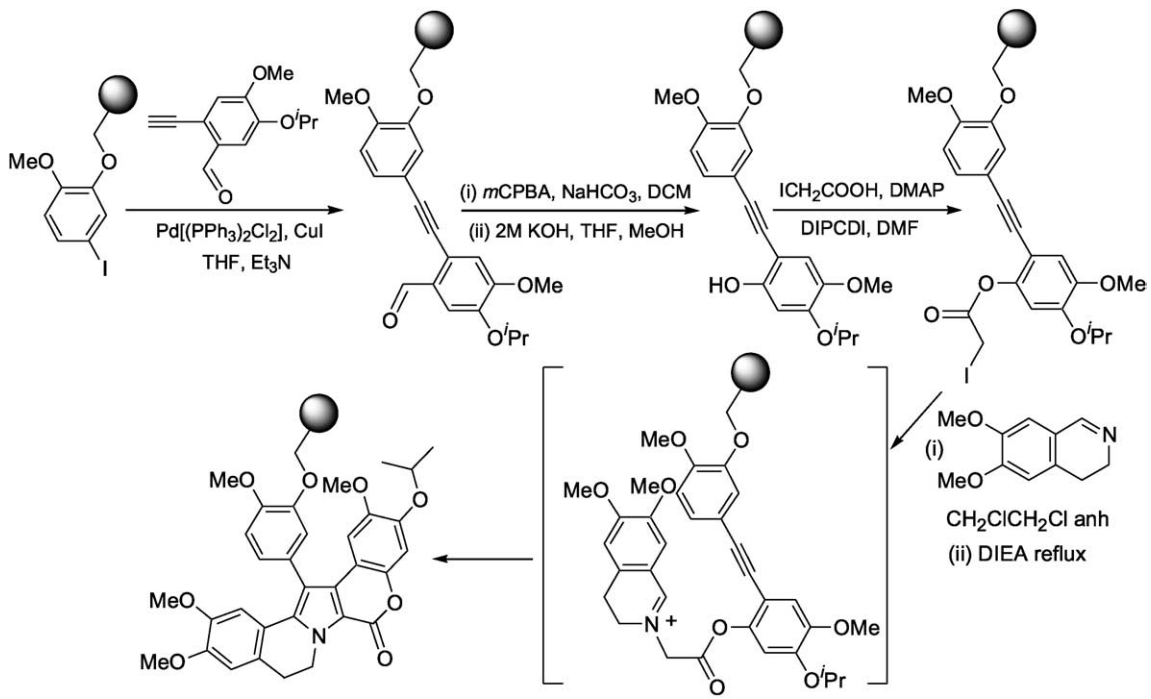

Figure 3. Solid-phase synthesis of lamellarins.

desired. Other MeO groups were already introduced into the building blocks. For the Wang resin, the scheme outlined in Figure 3 was followed, starting with a functionalization of 0.86 mmol/g. The process is similar, with minor modifications, to that followed for the solid-phase preparation using Merrifield-OH.[14]

Cleavages were performed with the reagents discussed above. As expected, cleavage with $ZnBr_2$ (4 equiv.) in dry DCM in the presence of AcBr (overnight, 10 equiv.) gave the acetyl derivatives corresponding to 3,3′-di-*O*-acetyllamellarin U. Cleavage with $AlCl_3$ in dry DCM (10 equiv.) produced mainly lamellarin U, accompanied by lamellarin L (free hydroxy at C11) and 12-*O*-demethoxylamellarin U (free hydroxy at C12). Although the main product was always Lamellarin U, the proportion of other derivatives can be increased by extending cleavage times (3–6 h). With a shorter cleavage time, the compound with an isopropoxy group at C3 was also obtained. This derivative (3-*O*-isopropyllamellarin U) was the main product when the cleavage was performed with TFA on Wang resin. In this cleavage, an unexpected derivative containing a chlorine atom at C2′ (2′-chloro-3-*O*-isopropyllamellarin U) was also obtained.[21] The purity of compounds obtained with the Merrifield resin is greater than when the Wang resin is used. Therefore, although cleavages carried out with Lewis acids can also be performed on Wang resin, the results obtained with Merrifield resins were better (Fig. 4).

In conclusion, the application of a number of Lewis acids as a cleavage/deprotection method in the solid-phase synthesis of organic molecules can be used as an important diversity point rendering several analogues, which, after purification, can be submitted for biological evaluation.

3. Experimental

3.1. General procedure

Hydroxymethyl poly(styrene-*co*-1% divinylbenzene) resin (Merrifield-OH, 100–200 mesh material, nominal loading: 0.68 mmol/g); chloromethyl poly(styrene-*co*-1% divinylbenzene)resin (Merrifield-Cl resin, 100–200 mesh material, nominal loading: 0.61 mmol/g), 4-hydroxymethylphenoxymethyl poly(styrene-*co*-1% divinylbenzene) resin (Wang resin, 100–200 mesh material, nominal loading: 0.86 mmol/g) were from NovaBiochem (Läufelfingen, Switzerland).

Tetrahydrofuran (THF) was freshly distilled from sodium/benzophenone. Dichloromethane was distilled from calcium hydride prior to use. DMF (99.99% anhydrous) was purchased from SDS and used as received.

^{1}H NMR and heterocorrelations (600 MHz) spectra were recorded on Bruker spectrometer, ^{13}C NMR (100 MHz) spectra were recorded on a Varian Mercury 400 spectrometer. Chemical shift (δ) are expressed in parts per million downfield from $CDCl_3$ as internal standard.

Analytical HPLC was carried out on a Waters® 2695

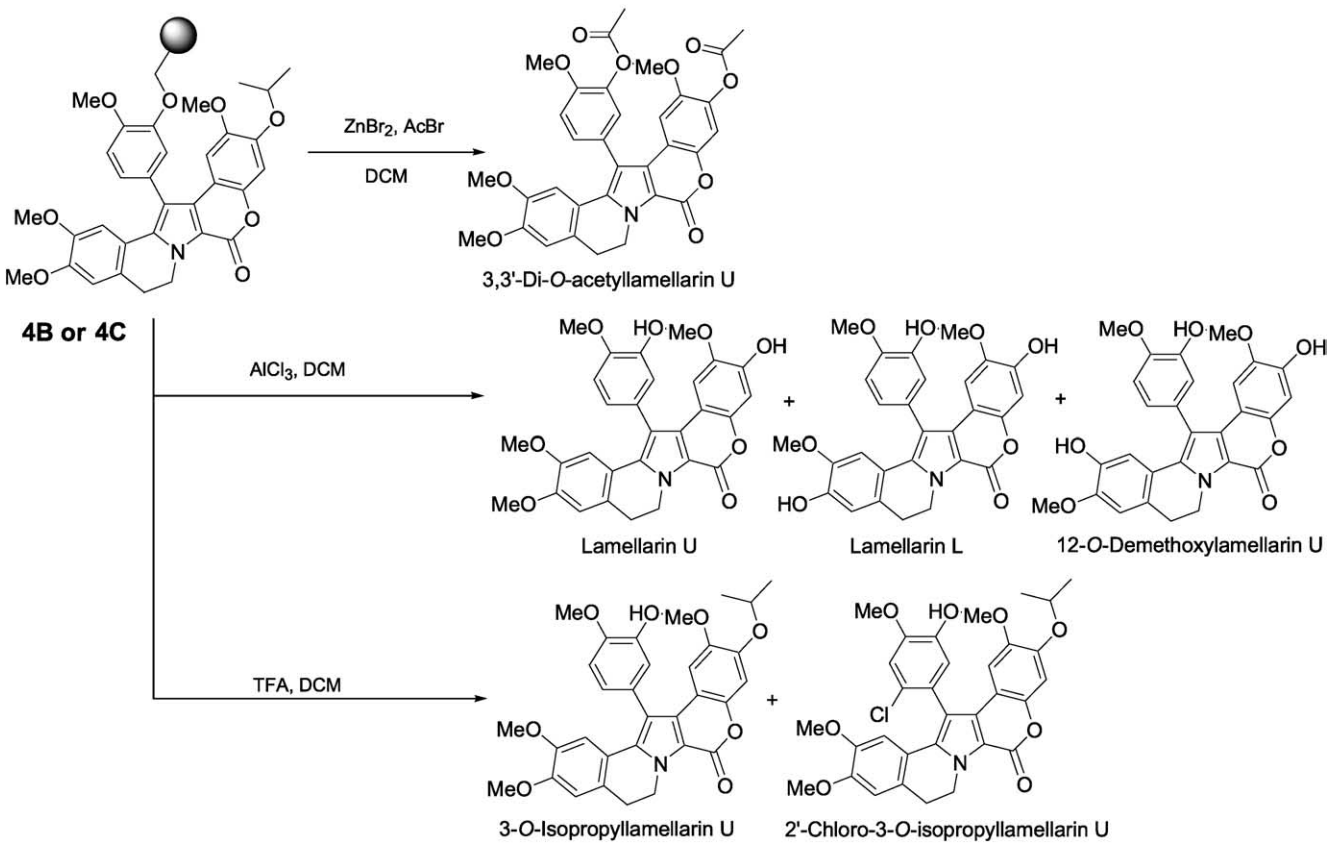

Figure 4. Lewis acid cleavage of solid-phase supported lamellarins.

Separations Module instrument, Water® 996 Photodiode Array Detector. UV detection from 210 to 500 nm and linear gradients of CH_3CN into H_2O were run at 1.0 ml/min flow rate from: 70:30 to 0:100 over 15 min, with a Symmetry® C-18 5.0 μm 4.6 mm×150 mm column. Preparative HPLC was carried out on a Waters® 600 Multisolvent Delivery System instrument, Waters® 2700 Sample Manager and a Water® 2487 Dual Absorbance UV Detector; detection at 277.0 and 315.0 nm. Linear gradients of CH_3CN into H_2O were run at 25.0 ml/min flow rate from: 70:30 to 40:60 over 60 min, with a Symmetry® C-8 5.0 μm 30 mm×100 mm column.

$APCI^+$-MS analysis of crude material and final compounds were performed in a Mass spectrometer VG Platform II, Micromass.

FAB^+ HR-MS were performed on a Autospec FAB+by Unidade de Espectrometría de Masas (Universidad de Santiago de Compostela).

3.2. Typical procedure for iodophenol anchorage to the hydroxy type resins (hydroxy Merrifield and Wang resins)

3.2.1. 5-Iodo-2-methoxyphenoxy-resin (1b, 1c). The procedure described by Spatola et al.[22] for the incorporation of Tyr trough a Mitsunobu reaction has been repeated here for the incorporation of 2-methoxy-5-iodophenol to Merrifield-OH and Wang resins. Merrifield-OH resin (1.0 g of 100–200 mesh material, 0.68 mmol/g loading) was washed with DCM (1×10 ml) and THF (1×10 ml). The dried resin was then treated with THF (15 ml), 2-methoxy-5-iodophenol (510 mg, 2.04 mmol, 3 equiv.), and triphenylphosphine (535 mg, 2.04 mmol, 3 equiv.), diisopropylethylamine (1.05 ml, 6.12 mmol, 9 equiv.) and the resulting mixture was stirred and cooled at 0 °C. Diethyl azodicarboxylate (320 μl, 2.04 mmol, 3 equiv.) was then added dropwise and the resulting mixture was stirred 3 h at room temperature. The solvent was then removed. The resin was washed with DMF, DCM, methanol and diethyl ether (5×15 ml each) and dried under reduced pressure. IR (KBr, cm^{-1}), in the IR spectrum it is possible to see the absence of at 3450 and 3580 cm^{-1} proper of hydroxyl groups of the resin; ^{13}C NMR-MAS (125 MHz) δ 149.8, 149.2, 130.1 (C-3), 122.7 (C-4), 113.7 (C-6), 82.2 (C-5), 55.9 (OCH_3).

3.2.2. 5-Iodo-2-methoxyphenoxy-resin (1a). Merrifield-Cl resin (1.0 g of 100–200 mesh material, 0.61 mmol/g loading) was washed with DCM (1×10 ml). The dried resin was then swelled in DMF (10 ml) for 30 min, then cesium 2-methoxy-5-iodo-phenolate (1.38 g, 3.6 mmol, 6 equiv.) was added in DMF (dry), finally KI (20 mg, 012 mmol, 0.2 eq.) was added. The resulting mixture was stirred at 50 °C for 24 h. It was then filtered and washed with DMF, DCM, MeOH and diethyl ether (5×15 ml each) and dried under reduced pressure. Spectroscopic data were similar as described in the previous example.

3.3. Typical procedure for the cleavage with anhydrous $SnCl_4$

3.3.1. 5-Iodo-2-methoxyphenol (3).[23] The resin **1A** or **1B** or **1C** (100 mg, 0.5 mmol/g theoretical loading) was swelled with dry DCM for 30 min, $SnCl_4$ (10 equiv.) was added and the reaction mixture was shaken under Ar overnight. After this time the resin was filtered off and washed with DCM (5×5 ml). The filtrates were washed with H_2O (6×15 ml), dried ($MgSO_4$ anhydrous), and concentrated under reduced pressure to give 5-iodo-2-methoxyphenol (12 mg of crude material, 70% of purity by HPLC). ^1H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, J=2.2 Hz, 1H, H-6), 7.15 (dd, J=8.4, 2.2 Hz, 1H, H-4), 6.59 (d, J=8.4 Hz, H-3), 5.60 (bs, 1H, OH), 3.87 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.6 (s), 146.5 (s), 129.0 (d, C-3), 123.4(d, C-4), 112.4 (d, C-6), 83.0 (s, C-5), 56.0 (q, OCH_3).

3.4. Typical procedure for the cleavage with anhydrous $ZnBr_2$

3.4.1. 5-Iodo-2-methoxyphenol (3). The resin **1A** or **1B** or **1C** (100 mg, 0.5 mmol/g theoretical loading) was swelled for 30 min with dry DCM 1 ml). After this time anhydrous $ZnBr_2$ (4 equiv.) were added and the reaction mixture was then shaken under Ar for 4 h at room temperature. The resin was filtered off and washed with DCM (5×5 ml). The filtrates were washed with aq. 5% $NaHCO_3$ (5×15 ml), 2 M HCl (3×15) and brine (3×15 ml). The organic solution was dried ($MgSO_4$ anhydrous), filtered and concentrated under reduced pressure to give 5-iodo-2-methoxyphenol (10.5 mg, 84%); CIMS m/z (relative intensity): 250 ([M^+],100). Spectroscopic data were the same as in the example above.

3.4.2. 5-Iodo-2-methoxyphenol acetate (2). The resin **1A** or **1B** or **1C** (100 mg, 0.5 mmol/g theoretical loading) was swelled for 30 min with dry DCM (1 ml). After this time anhydrous $ZnBr_2$ (4 equiv.) and AcBr (10 equiv.) were added and the reaction mixture was then shaken under Ar overnight at room temperature. The resin was filtered off and washed with DCM (5×5 ml). The filtrates were washed with aq. 5% $NaHCO_3$ (5×15 ml), 2 M HCl (3×15) and brine (3×15 ml). The organic solution was dried ($MgSO_4$ anhydrous), filtered and concentrated under reduced pressure to give 5-iodo-2-methoxyphenol acetate (13 mg, 89%). ^1H NMR (200 MHz, $CDCl_3$) δ 7.49 (dd, J=8.8, 2.0 Hz, 1H, H-4), 7.33 (d, J=2.0 Hz, 1H, H-6), 6.72 (d, J=8.8 Hz, H-3), 3.81 (bs, 3H, OCH_3), 2.30 (s, 3H, $COCH_3$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.5 (s, C=O), 151.3 (s, C-2), 140.4 (s, C-1), 135.6 (d, C-4), 131.5 (d, C-6), 114.2 (d, C-3), 81.3 (s, C-5), 56.0 (q, OCH_3), 20.6 (q, $COCH_3$).

3.4.3. 3,3′-Di-*O*-acetyllamellarin U. The resin **4B** (180 mg, 0.48 mmol/g theoretical loading) was swelled for 30 min with dry DCM (1 ml). After this time anhydrous $ZnBr_2$ (225 mg, 4 equiv.) and AcBr (111 mg, 10 equiv.) were added and the reaction mixture was then shaken under Ar overnight at 25 °C. The resin was filtered off and washed with DCM (5×5 ml). The filtrates were washed with aq. 5% $NaHCO_3$ (5×15 ml), 2 M HCl (3×15) and brine (3×15 ml). The organic solution was dried ($MgSO_4$ anhydrous), filtered and concentrated under reduced pressure. The crude was analyzed by HPLC-MS [C18-$APCI^+$ using H_2O (5 mM $AcNH_4$): acetonitrile gradient 30–100% acetonitrile in 15 min]: retention time 9.73 min, molecular weight 599.58; found 600.6 $[M+H]^+$. MSMS (Q-TOF) calcd 599.18; found 600.20 $[M+H]^+$, 557, 22

$[(M+H)^+-C_2H_2O]$, 515.29 $[(M+H)^+-2\times C_2H_2O]$. The crude product was purified by HPLC and the 3,3′-di-*O*-acetyllamellarin U (4 mg, 8.5% overall yield) was obtained. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30 (dd, J=8.2, 2 Hz, 1H, H-6′), 7.22 (d, J=2 Hz, 1H, H-2′), 7.12 (d, J=8.2 Hz, 1H, H-5′), 7.06 (s, 1H, H-4), 6.74 (s, 1H, H-10), 6.72 (s, 1H, H-1), 6.62 (s, 1H, H-13), 4.95 (m, 1H, H-8), 4.64 (m, 1H, H-8), 3.87 (s, 6H, C^{12}-OCH_3 and $C^{4'}$-OCH_3), 3.44 (s, 3H, C^2-OCH_3), 3.40 (s, 3H, C^{11}-OCH_3), 3.15 (m, 1H, H-9), 3.05 (m, 1H, H-9), 2.29 (s, 3H, C^3OOCCH_3), 2.27 (s, 3H, $C^{3'}$OOCCH_3); ^{13}C NMR (150 MHz, $CDCl_3$)[24] δ 168.8 (C^3OO*C*CH_3), 168.3 ($C^{3'}$OO*C*CH_3), 151.2 (C-4′), 149.0 (C-12), 147.5 (C-11), 147.4 (C-2), 144.8 (C-3), 140.7 (C-3′), 138.7 (C-4a), 136.1 (C-13b), 129.5 (C-6′), 127.7 (C-1′), 127.4 (C-14a), 126.4 (C-9a), 125.0 (C-2′), 119.8 (C-13a), 116.1 (C-14b), 114.1 (C-14), 112.5 (C-5′), 111.8 (C-4), 110.9 (C-10), 108.5 (C-13), 105.4 (C-1), 56.0 ($C^{4'}$-O*C*H_3), 56.0 (C^{12}-O*C*H_3), 55.6 (C^2-O*C*H_3), 55.2 (C^{11}-O*C*H_3), 42.5 (C-8), 28.7 (C-9), 20.0 ($C^{3'}$OOC*C*H_3), 20.0 (C^3OOC*C*H_3). (+)-HRMS *m*/*z* 599.1789 (calcd for $C_{33}H_{29}NO_{10}$ $[M]^+$ 599.1791, Δ +0.4 ppm).

3.5. Typical procedure for $AlCl_3$ cleavage

3.5.1. 5-Iodo-2-methoxyphenol (3). The resin **1A** or **1B** or **1C** (100 mg, 0.5 mmol/g theoretical loading) was swelled with dry DCM for 30 min, $AlCl_3$ (10 equiv.) was added and the reaction mixture was shaken under Ar for 3 h. After this time the resin was filtered off and washed with DCM (5×5 ml). The filtrates were washed with a sat. aq. solution of NH_4Cl (1×15 ml) and H_2O (6×15 ml), dried ($MgSO_4$ anhydrous), and concentrated under reduced pressure to give 5-iodo-2-methoxyphenol (11 mg, 88%). Spectroscopic data were the same as in the example above.

3.5.2. Deprotected lamellarins. The resin **4B** (220 mg, 0.44 mmol/g loading) was swelled in dry DCM (3 ml) for 30 min and $AlCl_3$ (220 mg, 1.65 mmol, 15 equiv.) was added. The reaction mixture was stirred in a vibromatic shaker at 25 °C for 6 h. It was then filtered and washed with DCM, AcOEt and MeOH (5×10 ml, each), the organic solvent was evaporated. The residue was taken with a sat. aq. solution of NH_4Cl and extracted with ethyl acetate (5×20 ml), then washed with brine (1×30 ml). The organic fraction was dried and evaporated. The HPLC/MS [C18-APCI$^+$ using H_2O (5 mM $AcNH_4$): acetonitrile gradient 30–100% acetonitrile in 15 min] shown three lamellarin derivatives: lamellarin U, retention time 8.0 min, calcd 515.16; found 516.19 $[M+H]^+$; demethyllamellarin U, retention time 6.9 min, calcd 501.14; found 502.15 $[M+H]^+$, and lamellarin L, retention time 6.6 min, calcd 501.14; found 502.15 $[M+H]^+$. The crude product was purified by HPLC. Lamellarin U (4.5 mg, 9.2%), demethyl-lamellarin L (1 mg, 2.0%), and lamellarin L (1.5 mg, 3.1%) were obtained.

3.5.3. Lamellarin U. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.11 (d, J=1.9 Hz, 1H, H-2′), 7.02 (d, J=8.2 Hz, 1H, H-5′), 6.99 (dd, J=8.2, 1.9 Hz, 1H, H-6′), 6.94 (s, 1H, H-4), 6.73 (s, 1H, H-10), 6.70 (s, 1H, H-13), 6.69 (s, 1H, H-1), 5.69 (s, 1H, C3-O*H*), 5.66 (s, 1H, C3′-O*H*), 4.80 (m, 1H, H-8), 4.72 (m, 1H, H-8), 3.95 (s, 3H, $C^{4'}$-OCH_3), 3.87 (s, 3H, C^{11}-OCH_3), 3.51 (s, 3H, C^2-OCH_3), 3.37 (s, 3H, C^{12}-OCH_3); 3.08 (dd, J=6.6, 5.7 Hz, 2H, H-9); ^{13}C NMR (150 MHz, $CDCl_3$)[23] δ 151.4 (C-3′), 149.3 (C-11), 147.9 (C-12), 146.8 (C-4′), 145.8 (C-4a), 143.6 (C-2), 136.3 (C-13b), 129.2 (C-1′), 128.6 (C-14a), 127.1 (C-9a), 123.4 (C-6′), 120.5 (C-13a), 117.4 (C-2′), 114.9 (C-14), 111.1 (C-5′), 110.9 (C-10), 110.7 (C-14b),108.8 (C-13), 104.1 (C-1), 103.3 (C-4), 56.2 ($C^{4'}$-O*C*H_3), 55.8 (C^{11}-O*C*H_3), 55.5 (C^2-O*C*H_3), 55.0 (C^{12}-O*C*H_3), 42.8 (C-8), 28,6 (C-9). (+)-HRMS *m*/*z* 516.1683 (calcd for $C_{29}H_{26}NO_8$ $[M+H]^+$ 516.1166, Δ −4.8 ppm).

3.5.4. Lamellarin L. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.11 (d, J=1.9 Hz, 1H, H-2′), 7.02 (d, J=8.3 Hz, 1H, H-5′), 6.98 (dd, J=8.3, 1.9 Hz, 1H, H-6′), 6.93 (s, 1H, H-4), 6.79 (s, 1H, H-10), 6.68 (s, 1H, H-1), 6.67 (s, 1H, H-13), 5.70 (bs, 2H), 5.60 (bs, 1H), 4.78 (m, 1H, H-8), 4.70 (m, 1H, H-8), 3.96 (s, 3H, $C^{4'}$-OCH_3), 3.50 (s, 3H, C^2-OCH_3), 3.41 (s, 3H, C^{12}-OCH_3), 3.01 (m, 2H, H-9); ^{13}C NMR (150 MHz, $CDCl_3$)[23] δ 146.4 (C-3), 146.3 (C-3′), 146.2 (C-4′), 145.7 (C-11), 145.1 (C-12), 143.2 (C-2), 135.4 (C-13b), 128.3 (C-1′), 128.2 (C-14a), 127.4 (C-9a), 122.9 (C-6′), 119.8 (C-13a), 117.3 (C-2′), 114.0 (C-10), 111.9 (C-5′), 108.4 (C-13), 104.1 (C-1), 103.5 (C-4), 56.2 ($C^{4'}$-O*C*H_3), 55.5 (C^2-O*C*H_3), 55.1 (C^{12}-O*C*H_3), 42.2 (C-8), 28.2 (C-9). (+)-HRMS *m*/*z* 502.1503 (calcd for $C_{28}H_{24}NO_8$ $[M+H]^+$ 502.1502, Δ −0.1 ppm).

3.5.5. Demethyllamellarin U. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.04 (d, J=2.0 Hz, 1H, H-2′), 7.00 (d, J=8.0 Hz, 1H, H-5′), 6.94 (dd, J=8.0, 2.0 Hz, 1H, H-6′), 6.92 (s, 1H, H-4), 6.74 (s, 1H, H-13), 6.73 (s, 1H, H-10), 6.57 (s, 1H, H-1), 5.68 (s, O*H*), 5.65 (s, O*H*), 5.36 (s, O*H*), 4.72–4.80 (m, 2H, H-8), 3.97 (s, 3H, $C^{4'}$-OCH_3), 3.89 (s, 3H, C^{11}-OCH_3), 3.49 (s, 3H, C^2-OCH_3), 3.07 (m, 2H, H-9); ^{13}C NMR (150 MHz, $CDCl_3$)[23] δ 146.3 (C-11), 146.2 (C-4′) 146.2 (C-3), 145.2 (C-4a), 144.9 (C-3′), 143.8 (C-12), 142.9 (C-2), 135.2 (C-13b), 128.1 (C-14a), 126.1 (C-9a), 122.8 (C-6′), 120.6 (C-13a), 117.1 (C-2′), 115.7 (C-14), 113.1 (C-14b), 112.1 (C-13), 111.2 (C-5′), 110.3 (C-10), 104.1 (C-1), 103.2 (C-4), 55.9 ($C^{4'}$-O*C*H_3), 55.7 (C^{11}-O*C*H_3), 55.3 (C^2-O*C*H_3), 42.3 (C-8), 28.8 (C-9). (+)-HRMS *m*/*z* 502.1508 (calcd for $C_{28}H_{24}NO_8$ $[M+H]^+$ 502.1502, Δ −1.1 ppm).

3.6. Typical procedure for TFA cleavage of Wang resin

3.6.1. 5-Iodo-2-methoxyphenol (3). A solution of TFA in DCM (1:1, 2 ml) was added to the Wang conjugate phenol **1C** resin (56 mg, 0.71 mmol/g theoretical loading) and the mixture was shaken for 2 h at room temperature. The resulting suspension was filtered of, the same acid solution was added and the mixture was shaken for 2 h. This process was repeated two times. Finally the resin was washed several times with DCM. The filtrates were washed with H_2O (3×25 ml), dried ($MgSO_4$ anhydrous), and concentrated under reduced pressure to give a very clean 5-iodo-2-methoxyphenol (8 mg, 81%). Spectroscopic data were the same as in the example above.

3.6.2. Hydroxy protected lamellarins. Following the general procedure of cleavage with TFA described above, from **4C** (300 mg) a reaction crude was obtained. The HPLC/MS [C18-APCI$^+$ using H_2O (5 mM $AcNH_4$): acetonitrile gradient 30–100% acetonitrile in 15 min]

shown two different lamellarins derivatives: 2′-chloro-3-*O* isopropyllamellarin U retention time 11.07 min, calcd 591.17; found 592.23 [^{35}Cl M+H]$^+$, 594.23 [^{37}Cl M+H]$^+$, 3-*O*-isopropyllamellarin U retention time 11.3 min, calcd 557.20; found 558.30 [M+H]$^+$. The crude product was purified by HPLC. 2′-Chloro-3-*O*-isopropyllamellarin U (2 mg, 2% overall yield), and 3-*O*-isopropyllamellarin U (8.5 mg, 9%, overall yield) were obtained.

3.6.3. 3-O-Isopropyllamellarin U. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.11 (d, J=2.0 Hz, 1H, H-2′), 7.01 (d, J=8.3 Hz, 1H, H-5′), 6.99 (dd, J=8.3, 2.0 Hz, 1H, H-6′), 6.90 (s, 1H, H-4), 6.73 (s, 1H, H-10), 6.72 (s, 1H, H-13), 6.71 (s, 1H, H-1), 5.68 (bs, 1H, O*H*), 4.83 (m, 1H, H-8), 4.74 (m, 1H, H-8), 4.52 (hept, 1H, C^3-OC*H*$(CH_3)_2$), 3.94 (s, 3H, C$^{4'}$-OC*H*$_3$), 3.87 (s, 3H, C^{11}-OC*H*$_3$), 3.45 (s, 3H, C^2-OC*H*$_3$), 3.37 (s, 3H, C^{12}-OC*H*$_3$), 3.09 (m, 2H, H-9), 1.37 (d, 6H, C^3-OCH(C*H*$_3$)$_2$); ^{13}C NMR (150 MHz, $CDCl_3$)[23] δ 148.8 (C-11), 147.4 (C-12), 147.1 (C-3), 146.4 (C-2), 146.3 (C-4′), 146.1 (C-3′), 135.8 (C-13b), 128.6 (C-1′), 128.1 (C-14a), 126.5 (C-9a), 122.9 (C-6′), 120.1 (C-13a), 117.3 (C-2′), 114.7 (C-14) 111.2 (C-5′),110.9 (C-10), 110.3 (C-14b), 108.8 (C-13), 105.1 (C-1), 103.5 (C-4), 71.4 (C^3-O*C*H$(CH_3)_2$), 56.2 (C$^{4'}$-O*C*H$_3$), 55.8 (C^{11}-O*C*H$_3$), 55.4 (C^2-O*C*H$_3$), 55.0 (C^{12}-OC*H*$_3$), 42.3 (C-8), 28.6 (C-9), 21.7 (C^3-OCH(*C*H$_3$)$_2$). (+)-HRMS *m*/*z* 557.2067 (calcd for $C_{32}H_{31}NO_8$ [M]$^+$ 557.2050, Δ −3.0 ppm).

The presence of the unexpected 2′-chloro-3-*O*-isopropyllamellarin U was confirmed by ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.11 (s, 1H, H-6′), 7.07 (s, 1H, H-3′) 6.90 (s, 1H, H-4), 6.75 (s, 1H, H-10), 6.62 (s, 1H, H-13), 6.56 (s, 1H, H-1), 5.61 (bs, 1H, O*H*), 4.93 (m, 1H, H-8), 4.66 (m, 1H, H-8), 4.51 (hept, 1H, C^3-OC*H*$(CH_3)_2$), 3.96 (s, 3H, C$^{4'}$-OC*H*$_3$), 3.87 (s, 3H, C^{11}-OC*H*$_3$), 3.48 (s, 3H, C^2-OC*H*$_3$), 3.42 (s, 3H, C^{12}-OC*H*$_3$), 3.09 (m, 2H, H-9) 1.38 (d, 6H, C^3-OCH(C*H*$_3$)$_2$); ^{13}C NMR (150 MHz, $CDCl_3$)[23] δ 148.9 (C-11), 148.4 (C-5′), 147.1 (C-3), 147.4 (C-12), 146.9 (C-4′), 146.5 (C-2), 145,8 (C-4a), 135.9 (C-13b), 128.3 (C-14a), 126.6 (C-9a), 126.2 (C-1′), 119.9 (C-13a), 118.6 (C-3′)[24] 112.0 (C-6′),[25] 110.9 (C-10), 109.9 (C-14b), 108.2 (C-13), 104.7 (C-1), 103.3 (C-4), 71.3 (C^3-O*C*H$(CH_3)_2$, 56.4 (C$^{4'}$-O*C*H$_3$), 55.7 (C^{11}-O*C*H$_3$), 55.4 (C^2-O*C*H$_3$), 55.0 (C^{12}-OC*H*$_3$), 45.4 (C-8), 28.6 (C-9), 21.6 (C^3-OCH(*C*H$_3$)$_2$). (+)-HRMS *m*/*z* 592.1751 (calcd for $C_{32}H_{31}NO_8Cl$ [M+H]$^+$ 592.1738, Δ −2.2 ppm.

Acknowledgements

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25. Assignment may be reversed.

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Synthesis of a PNA-encoded cysteine protease inhibitor library☆

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Abstract—Peptide nucleic acids (PNAs) have been used to encode a combinatorial library whereby each compound is labeled with a PNA tag which reflects its synthetic history and localizes the compound upon hybridization to an oligonucleotide array. We report herein the full synthetic details for a 4000 member PNA-encoded library targeted towards cysteine protease.

1. Introduction

Microarray based technologies have proven to be a powerful analytical tool for their ability to consider large numbers of data points in a miniaturized format. Such microarrays can be prepared by several techniques including photolithography, contact printing and inkjet to generate arrays with densities ranging from 1000 to 500,000 features per square centimeters.[1] The success of microarrays in genomic analysis[2] has prompted researchers in other areas to adopt this format. To date, a number of chemistries have been developed to derivatize glass surfaces for protein,[3] oligosaccharide[4] and small molecule microarrays.[5] A strong motivation for developing small molecule microarrays is to miniaturize high throughput screening. Considering that an arrayed glass slide prepared by contact printing can present 10,000 analytes to be screened in 50 μl, this represent a 1000 fold miniaturization over the 1536 microtiter plate format. Another application of small molecule microarrays is to measure the functional activity of important enzymes[6] such as kinases or proteases from complex biological mixtures. Recently, peptide nucleic acid (PNA) tags have been used to label proteins[7] and encode small molecules[8] such that hybridization to an oligonucleotide microarray (Fig. 1) localizes the tagged entity to its preprogrammed location. An added advantage of using this strategy in combinatorial synthesis is that libraries synthesized in a split and mix format[9] can be decoded in a single operation with no redundant library members. Furthermore, the library can be screened in solution prior to hybridization which should reduce nonspecific interactions and allows the separation of bound and unbound ligand by size exclusion. This latter point is important since, by including a label with the PNA tag, the isolated ligands can be identified directly upon hybridization thereby avoiding the necessity to label the target.[8]

The library reported herein was targeted towards cysteine proteases for their well know involvement in a number of diseases[10] including tumor growth,[11] osteoporosis,[12] inflammation, neurodegenerative diseases[13] apoptosis misregulation,[14] and infectious diseases such malaria,[15] African sleeping sickness and Chaga's disease. It is important to note that in at least eight cases tested, the PNA tag and its linker did not interfere with the biological activity of the inhibitor.

2. Results and discussion

2.1. Synthetic strategy

The general structure of the PNA-encoded library **1** is

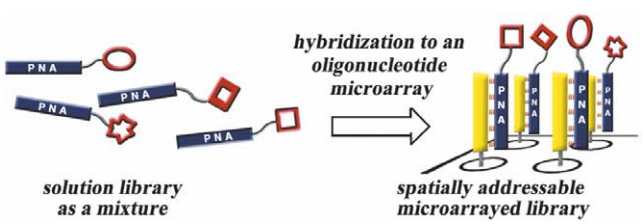

Figure 1. Hybridization of PNA-encoded library to an oligonucleotide microarray.

☆ Supplementary data associated with this article can be found in the online version, at doi: 10.1016/j.tet.2004.05.107

Keywords: Microarrays; Combinatorial chemistry; Protease inhibitors; Peptide nucleic acid.
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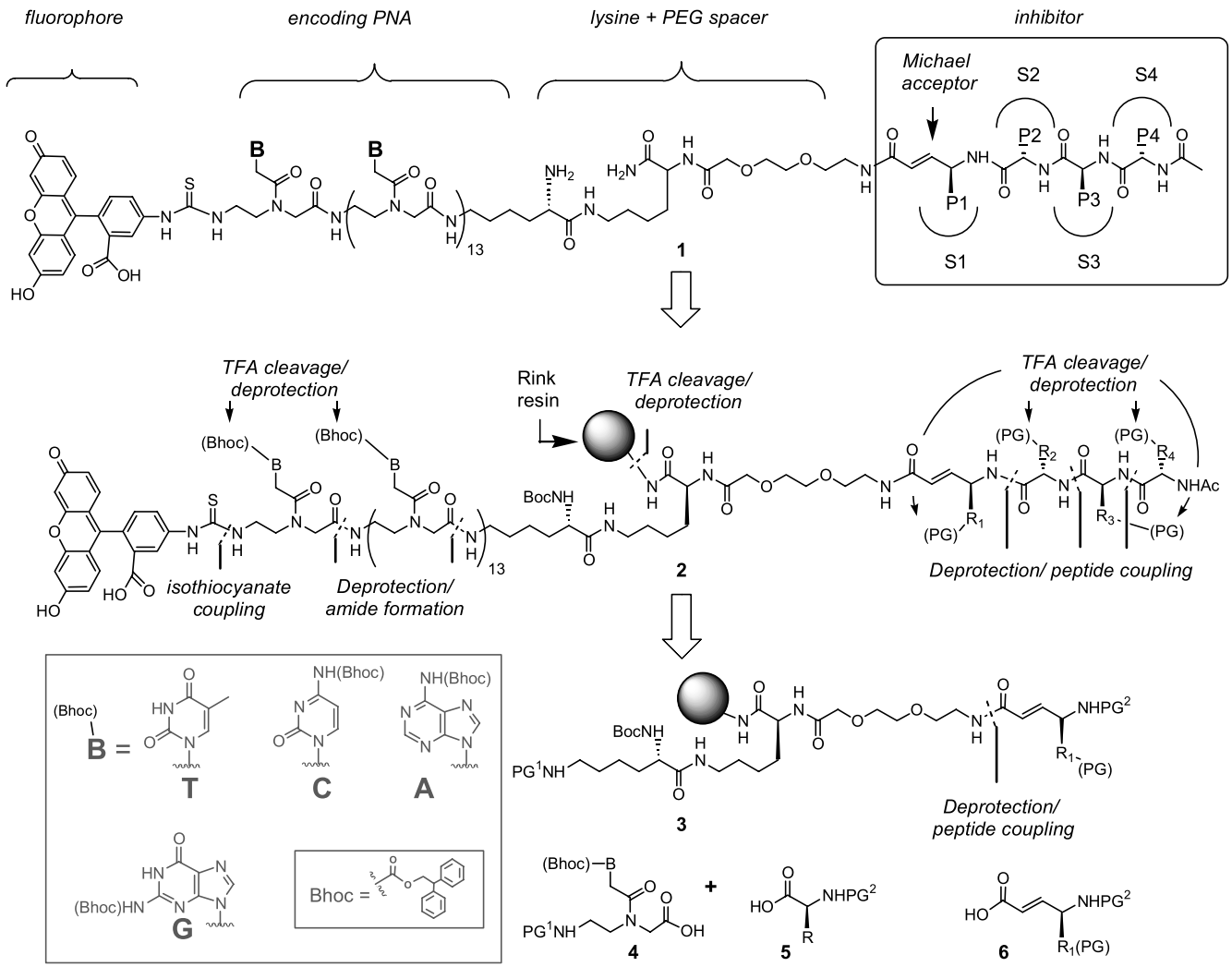
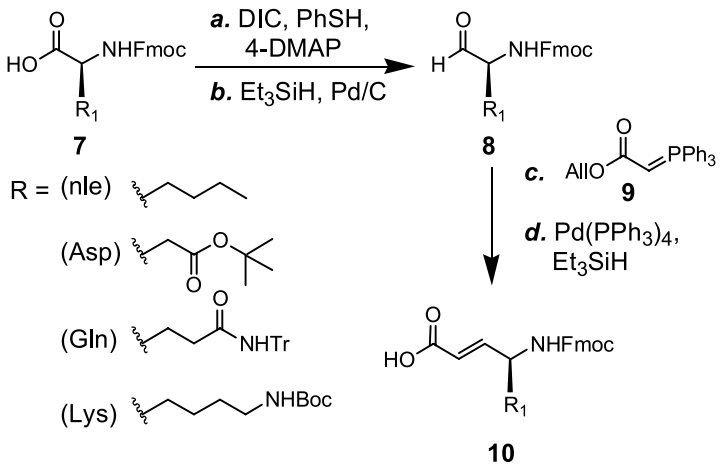


Figure 2. Design and retrosynthetic analysis of PNA-encoded library.

shown in Figure 2. The inhibitor is based on an acrylate functionality[16] that upon binding in the enzymatic pocket can be subject to a Michael addition of cysteine's nucleophilic thiol. The use of amino acids as the source of diversity was designed to maximize the potential of finding a protease–ligand interaction. A spacer (ca. 20 Å) containing a bis-ethyleneglycol moiety and a free amino group was included between the inhibitor and the PNA to insure good water solubility and reduce the risk of aggregation of the final library. Fluorescein was selected as the label based on the fact that it is excited with a green argon laser available in a number of microarray scanners, it can be potentially amplified with anti-fluorescein antibodies and it can be easily introduced from the commercially available isothiocyanate (FITC). Library **1** was anticipated to come from polymer-bound library **2** using a Rink acid labile linker, acid labile protecting groups for the PNAs' nucleobases as well as for the amino acid side chains. Two orthogonal protecting groups (PG^1 and PG^2) are required to carry out the alternative oligomerization of the PNA and inhibitor from **3** using the suitably protected monomers **4**–**6**. For this purpose, Fmoc and Alloc protecting group were selected for their mutual orthogonality and the accessibility of the corresponding monomers **4**–**6**.

2.2. Alloc-protected PNA and Fmoc-protected inhibitor oligomer (PG1=Alloc, PG2=Fmoc)

The required Fmoc protected acrylic amino acids **10** were prepared from commercially available Fmoc-protected amino acids **7** as shown in Scheme 1. Thus, esterification of the acids **7** (Nle, Asp, Gln, Lys) with ethane thiol afforded the corresponding thioesters which were reduced to aldehydes **8** with palladium on charcoal and triethyl silane in good yield.[17] The aldehydes **8** were then condensed with commercially available ylide **9** to afford the allyl acrylic esters which were deprotected with palladium-catalyzed allyl transfer to yield the desired Fmoc protected acrylic amino acids **10**. The required Alloc-protected PNA monomers **14** were most conveniently obtained by exchanging the Fmoc protecting group from commercially available PNA monomers **11** while temporarily loading them on a 2-chlorotrityl resin (Scheme 2) thereby avoiding all purifications and workups. Thus, acids **11** were coupled to 2-chlorotrityl resin to obtain polymer-bound esters **12**. Fmoc deprotection with DBU[18] and reprotection with allyl chloroformate yielded the Alloc protected esters **13** which were released from the resin using acetic acid in trifluoroethanol/dichloromethane[19] to obtained suitably

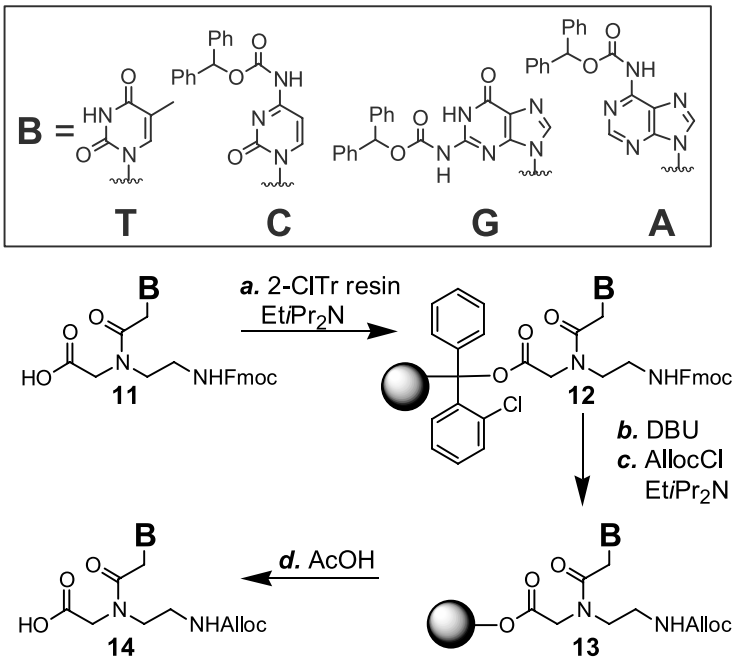


Scheme 1. Synthesis of Fmoc protected acrylic amino acids **10**. Reagents and conditions: (a) **7** (1.0 equiv.), DIC (1.6 equiv.), PhSH (1.1 equiv.), 4-DMP (0.05 equiv.), CH_2Cl_2, 23 °C, 30 min, 94% for Asp, 88% for nle, 95% for Gln, 94% for Lys; (b) Et_3SiH (4.0 equiv.), 5% Pd/C (0.02 equiv.), CH_2Cl_2, 23 °C, 90 min, 77% for **8**-**Nle**, 87% for **8**-**Asp**, 94% for **8**-**Gln**, 80% for **8**-**Lys**; (c) **9** (1.6 equiv.), Et*i*Pr_2N (1.4 equiv.), toluene, 80 °C, 90 min, 86% for Nle, 83% for Asp, 90% for Gln, 834% for Lys; (d) Bu_3SnH (3.0 equiv.), $Pd(PPh_3)_4$ (0.1 equiv.), CH_2Cl_2, 23 °C, 90 min, 86% for **10**-**Nle**, 82% for **10**-**Asp**, 89% for **10**-**Gln**, 91% for **10**-**Lys**.

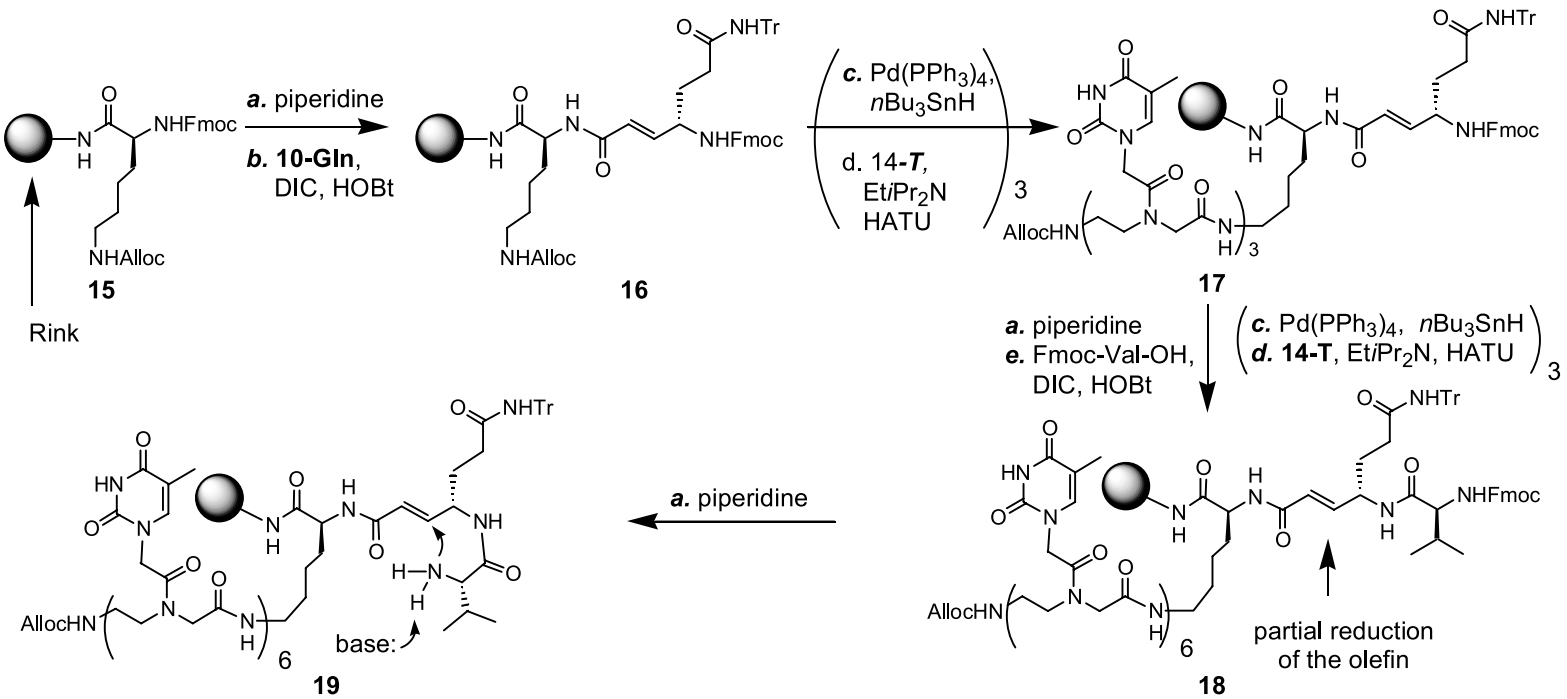
protected PNA monomer **14**. TFA cleavages with as little as 1% TFA in CH_2Cl_2 lead to partial Bhoc deprotection in 5 min. With the required building blocks at hand we turned our attention to the co-synthesis of the PNA and inhibitor. As shown in Scheme 3, Rink resin loaded with the orthogonally protected *N*-α-Fmoc-*N*-ε-Alloc-lysine (**15**) was substitute with the acrylic amino acid **10**-**Gln** to obtain resin **16**. The Alloc group was removed and a representative codon was introduced by reiteratively coupling PNA monomer **14**-**T** to obtain compound **17**. Sequential Fmoc deprotection, introduction of a second amino acid, Alloc deprotection and introduction of a second codon afforded compound **18** which was engaged in a third cycle. During this synthesis, two side reactions that could potentially compromise the purity of a final library were observed. First, during certain Alloc deprotection [$(Pd(PPh_3)_4$, *n*Bu_3SnH], partial reduction of the acrylate olefin was observed and second, basic conditions used in the Fmoc deprotection (piperidine or DBU) for compound **18** lead to partial piperazinone formation via intermediate **19**. With these results, we reasoned that it would be prudent to reverse the protecting group strategy since the Alloc deprotections may be carried out under acidic condition and would avoid the piperazinone formation and, the reduced number of Alloc deprotections should minimize the level of acrylate reduction.

2.3. Fmoc-protected PNA and Alloc-protected inhibitor oligomer (PG1=Fmoc, PG2=Alloc)

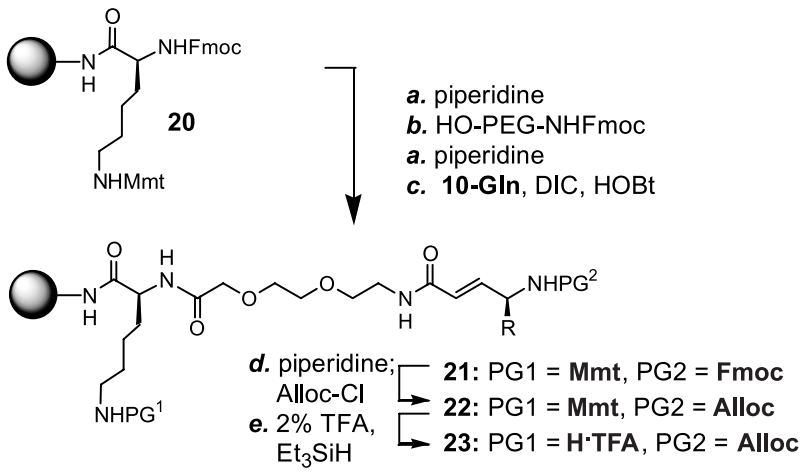
Rink resin loaded with the orthogonally protected



Scheme 2. Preparation of Alloc-protected PNA monomers **14**. Reagents and conditions: (a) **11** (1.0 equiv.), Et*i*Pr_2N (1.0 equiv.), resin (2.0 equiv.), CH_2Cl_2, 23 °C, 3 h; (b) DBU (1.0 equiv.) CH_2Cl_2, 23 °C, 5 min; (c) Alloc-Cl (3.0 equiv.), Et*i*Pr_2N (4.0 equiv.), CH_2Cl_2, 0 °C, 2×10 min; (d) AcOH–CF_3CH_2OH–CH_2Cl_2 (1:1:8), 60 min, precipitation in Et_2O, 60% for **14**-**T**, 46% for **14**-**C**, 61% for **14**-**G**, 58% for **14**-**A**, average purity >95%.

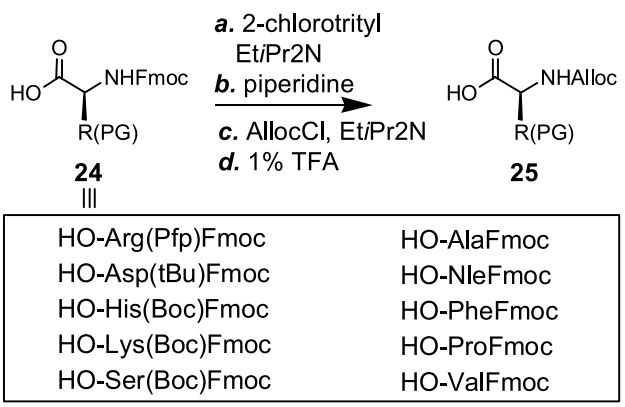


Scheme 3. Optimization of conditions for the co-synthesis of PNA and peptides using Alloc-protected PNA and Fmoc-protected amino acids. Reagents and conditions: (a) 20% piperidine, DMF, 23 °C, 2.5 min; (b) **10-Gln** (2.0 equiv.), DIC (4.0 equiv.), HOBt (4.0 equiv.), DMF, 23 °C, 3 h; (c) nBu$_3$SnH (10 equiv.), Pd(PPh$_3$)$_4$ (0.2 equiv.), CH_2Cl_2, 23 °C, 30 min; (d) **14-T** (4.0 equiv.), EtiPr$_2$N (4.0 equiv.), HATU (3.5 equiv.), DMF, 23 °C, 1 h.



Scheme 4. Preparation of Alloc-protected acrylamide **23**. Reagents and conditions: (a) 20% piperidine, DMF, 23 °C, 2.5 min; H·TFA/Alloc (2.0 equiv.), DIC (4.0 equiv.), HOBt (4.0 equiv.), DMF, 23 °C, 6 h; (c) **10-Gln** (2.0 equiv.), DIC (4.0 equiv.), HOBt (4.0 equiv.), DMF, 23 °C, 6 h; (d) 20% piperidine, DMF, 23 °C, 2.5 min; Alloc-Cl (4.0 equiv.), Et*i*Pr$_2$N (4.0 equiv.), DMF, 0 °C, 30 min; (d) 1% TFA, 5% Et$_3$SiH, CH_2Cl_2, 23 °C, 1 h.

N-α-Fmoc-*N*-ε-4-methyltrityl-lysine (**20**, Scheme 4) was substitute with the ethyleneglycol spacer followed by acrylic amino acid **10-Gln** to obtain resin **21**. The Fmoc group was exchanged for an Alloc and the methyltrityl was removed to obtain compound **23** as a TFA salt via **22**. The required Alloc-protected amino acids **25** with the appropriate side chain protecting groups could be readily obtained from the corresponding Fmoc protected amino acids **24** (Scheme 5) by temporarily loading them on a chlorotrityl resin. As for the PNA, this procedure avoided all work ups and purifications while delivering the amino acids with the appropriate side chain protecting groups. We then preceded to the synthesis of a set of representative PNA encoded inhibitors of which an example is Scheme 6. Thus, alternative addition of a PNA codon by reiterative Fmoc deprotection/coupling followed by Alloc deprotection and amino acid coupling led after labeling with FITC and cleavage from the resin to compound **26**. The use of triethyl silane[20] rather than tributyl tin hydride for the Alloc deprotection, while slower, proved to be more reliable and avoided all acrylate reduction problems. The final compound **26** was thus obtained from intermediate **20** in 40 steps. MALDI analysis of the final product shows the desired compound as a single major compound (Fig. 3). The relatively small number of truncated sequences reflects the efficiency of the well-established coupling procedures[21] and of the protecting group strategy.



Scheme 5. Exchange of protection groups for amino acid monomers. Reagents and conditions: (a) **24** (1.4 equiv.), Et*i*Pr$_2$N (2.0 equiv.), resin (1.0 equiv.) CH_2Cl_2, 23 °C, 3 h; (b) 20% piperidine in DMF, 23 °C, 10 min; (c) Alloc-Cl (4.0 equiv.), Et*i*Pr$_2$N (4.0 equiv.), CH_2Cl_2, 0 °C, 30 min; (d) 2.5% TFA in CH_2Cl_2, 23 °C, 30 min, 78% average yield, >95% purity by NMR.

2.4. Split and mix synthesis of the library

The codon system shown in Figure 4 was designed based on the following criteria: At least 2 base pair mismatches between each codon, no more than 6 consecutive purines and homogeneous hybridization properties with 4-letter codons at the extremities. The synthesis of the library was executed as shown in Scheme 7. The completion of each reaction was verified by mass spectroscopy analysis, a spectra of a representative pool is shown in Figure 5 (remaining spectra's are shown in the Supplementary Material). Thus, the four library pools (**23**) were encoded with their respective PNA sequences to obtain four pools of **27** which were mixed and subjected to a common Alloc deprotection before being split into ten new pools. To each

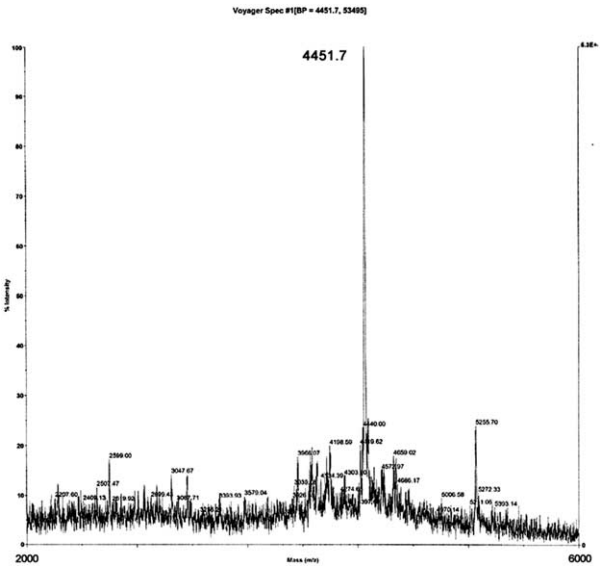
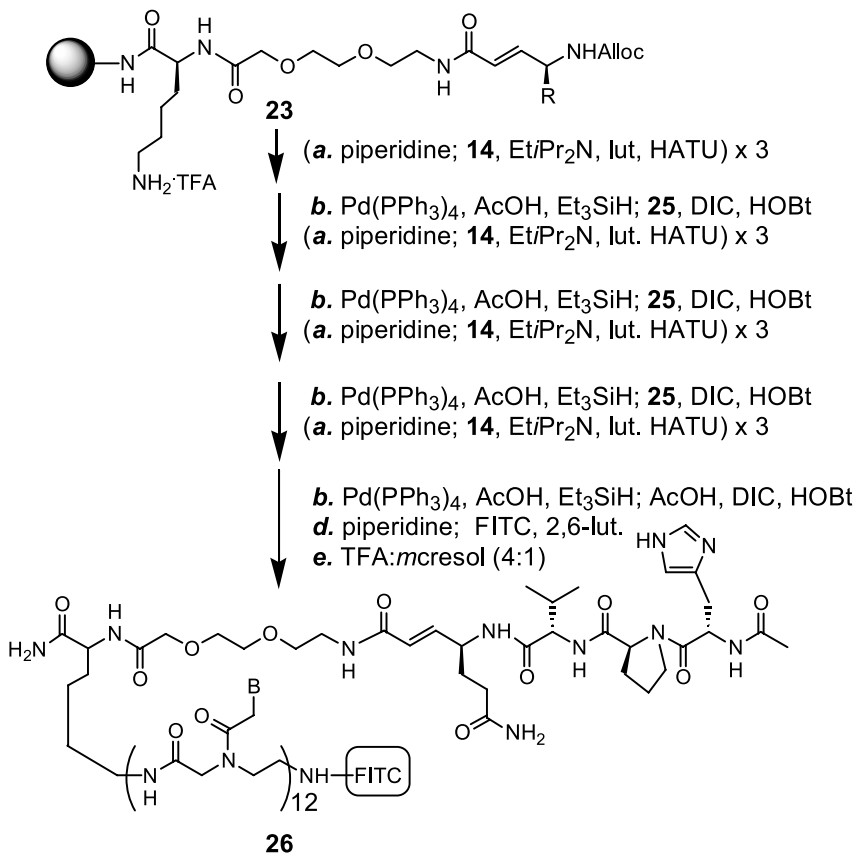


Figure 3. MALDI analysis of compound **26**.



Scheme 6. Optimized synthesis of PNA-encode inhibitor **26**. (a) 20% piperidine, DMF, 23 °C, 2.5 min; (b) **14**, Et*i*Pr$_2$N (4.0 equiv.), 2,6-lut. (6.0 equiv.), HATU (3.5 equiv.), DMF, 23 °C, 1 h; (c) Et_3SiH (10 equiv.), $Pd(PPh_3)_4$ (0.2 equiv.), AcOH (10 equiv.), DMF/CH_2Cl_2 (1/1), 23 °C, 30 min; **25** (4.0 equiv.), DIC (4.0 equiv.), HOBt (4.0 equiv.), DMF , 23 °C, 2 h; (d) 20% piperidine, DMF, 23 °C, 2.5 min; FITC (10 equiv.), lut. (10 equiv.), DMF, 23 °C 5 h; (e) TFA: *m*cresol (4:1), 23 °C, 2 h, Et_2O precipitation, centrifugation.

pool was added the second element of diversity followed by its respective PNA codon to obtain ten pools of **28** each containing 4 compounds. The pools were mixed deprotected and re-split into 10 new pools which were subjected to another round of diversification/encoding to yield ten pools of **29** each containing 40 compounds. While the identity of every component of a pool could no longer be identified by mass spectroscopy, the absence of lower molecular weight peaks suggests that all the steps thus far were very high yielding as was observed in a number of test cases such as compound **26**. The ten pools of **29** were once more mixed, deprotected, split and the last amino acid followed by the last codon was introduced to obtain 10 pools of **30** which

R^1	R^2	R^3	R^4
	Ala = TCC	CAA	CAAC
Asp = CCCA	Nle = CTC	ACA	ACAC
Gln = GGGT	Val = CCT	AAC	AACC
Nle = GGCA	Pro = TGG	CTT	CTTC
Lys = CCGT	Phe = GTG	TCT	TCTC
	His = GGT	TTC	TTCC
	Arg = AGC	ATG	ATGC
	Lys = GAC	TAG	TAGC
	Ser =CGA	GTA	GTAC
	Asp = CAG	AGT	AGTC

Figure 4. Sequence of the assigned codons.

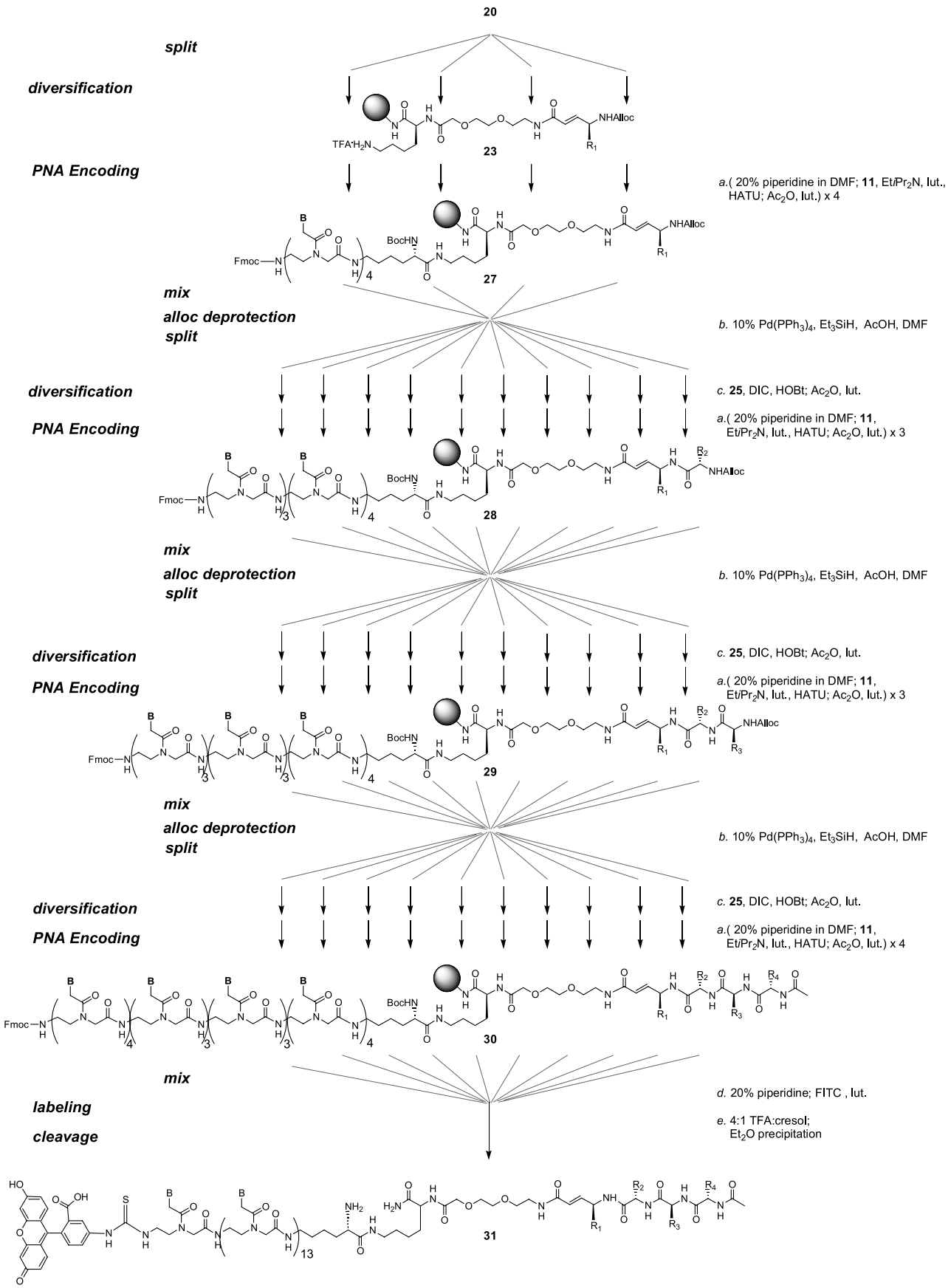
were combined. MALDI analysis was no longer useful to evaluate this last step as there are too many compounds in the mixture. The last Fmoc was removed and the library was labeled with fluorescein isothiocyanate (FITC). Finally, the library was cleaved as a mixture of 4000 compounds with TFA/cresol and precipitated in diethyl ether to obtain the library free of protecting group byproducts. Quantification of the fluorescein indicates a 32% recovery based on the loading of starting resin **20**.

A PNA-encoded library of 4000 compounds by split and mix synthesis was achieved. The use of this library to profile protease activity and identify inhibitors for subsequent biological chemistry study will be reported shortly. While the Alloc-protected PNA monomers proved to be less suitable than the corresponding Fmoc monomers for the present library, their chemistry should be applicable to other libraries and, by virtue of their stability to basic condition, further extend the scope of reactions to bring about molecular diversity with PNA encoding.

3. Experimental

3.1. General techniques

All reactions were carried out under a nitrogen atmosphere with dry, freshly distilled solvents under anhydrous



Scheme 7. PNA-encode split and mix synthesis of library **31**. Reagents and conditions: (a) 20% piperidine, DMF, 23 °C, 2.5 min; **14** (4.0 equiv.), Et*i*Pr$_2$N (4.0 equiv.), HATU (3.5 equiv.), 2,6-lut. (6.0 equiv.), DMF, 23 °C, 1 h; Ac_2O (4.0 equiv.), lut. (4.0 equiv.), DMF, 23 °C, 5 min; (b) Et_3SiH (10 equiv.), $Pd(PPh_3)_4$ (0.2 equiv.), AcOH (10 equiv.), DMF/CH_2Cl_2 (1/1), 23 °C, 30 min; (c) **25** (4.0 equiv.), DIC (4.0 equiv.), HOBt (4.0 equiv.), DMF, 23 °C, 2 h; Ac_2O (4.0 equiv.), lut. (4.0 equiv.), DMF, 23 °C, 5 min; (d) 20% piperidine, DMF, 23 °C, 2.5 min; FITC (10 equiv.), lut. (10 equiv.), DMF, 23 °C, 5 h; (e) TFA–cresol (4:1), 23 °C, 3 h; precipitated in Et_2O.

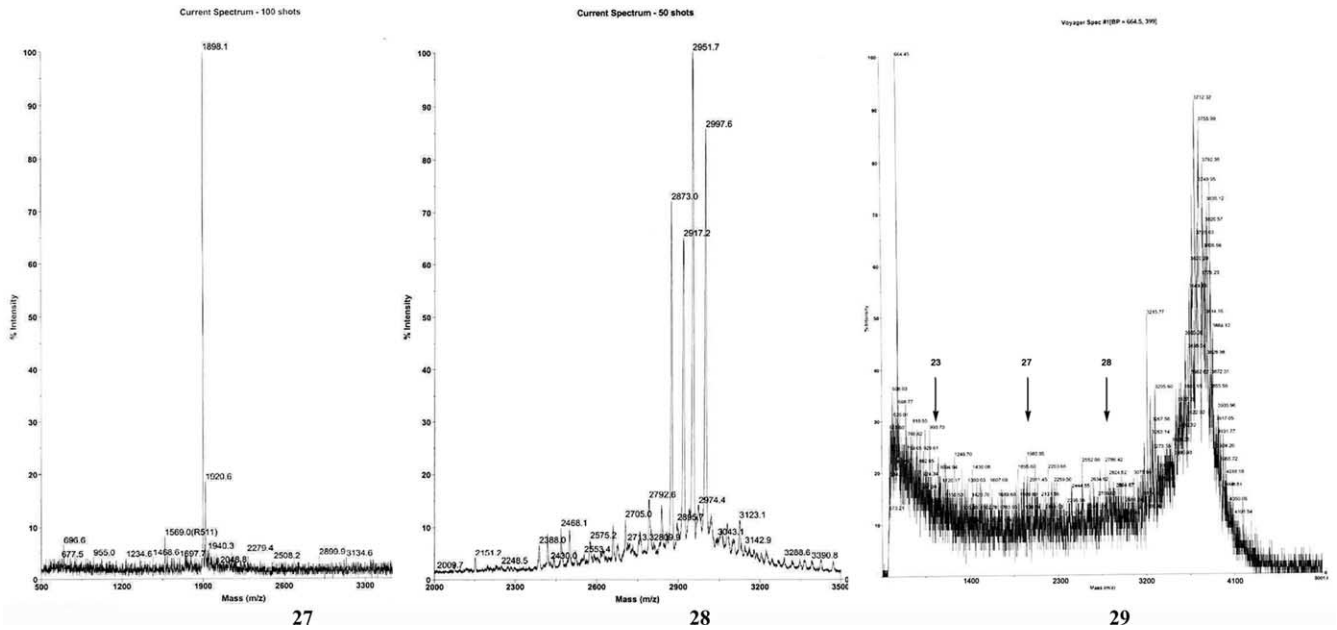


Figure 5. MALDI analysis of representative pools along the library synthesis.

conditions, unless otherwise noted. All solid phase reactions were carried out at ambient temperature unless specified otherwise. Tetrahydrofuran (THF), toluene and diethyl ether (Et_2O) were distilled from sodium–benzophenone, and methylene chloride (CH_2Cl_2) from calcium hydride. Anhydrous solvents were also obtained by passing them through commercially available activated alumina columns (Solv-Tek, Inc., VA). Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Substituted polystyrene resins (100–200 mesh, 1% DVB) and amino acids were purchased from Advanced Chemtech or Novabiochem. PNA monomers and polyethyleneglycol spacer were purchased from Applied Biosystems. Solution reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light for visualization agent and 10% ethanolic phosphomolybdic acid, ninhydrin or vanillin solution and heat, as developing agents. E. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash column chromatography. NMR spectra were recorded on Bruker Advance-400 instruments and calibrated using residual undeuterated solvent as an internal reference. Multiplicities were abbreviated as: s=singlet, d=doublet, t=triplet, q=quartet, qt=quintet, m=multiplet, b=broad. IR spectra were recorded on a Perkin–Elmer 1600 series FT-IR spectrometer. HRMS were recorded on a Bruker micro-TOF instrument (ESI) while MALDIs were performed on an Applied Biosystem Voyager.

3.2. Fmoc-protected acrylic amino acids 10

General procedure for the thioesterification. To a stirring solution of Fmoc protected amino acid **7** in CH_2Cl_2 (0.2–0.3 M) was added thioethanol (1.2 equiv.), followed by *N*,*N*-diisopropylcarbodiimide (1.6 equiv.) and 4-DMAP (0.05 equiv.). The reaction mixture was stirring for 25–30 min at 23 °C then loaded directly onto a silica gel column and eluted with EtOAc–hexanes.

3.2.1. Ethyl *N*-Fmoc-*O*-*t*Butyl-thioaspartate. 94% yield as viscous oil; R_f=0.75 (SiO_2, EtOAc–hexanes 1:1); IR (KBr): 3339.9, 2976.3, 1731.1, 1503.4, 1450.0 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.78 (2H, d, J=7.0 Hz, ArH), 7.68 (2H, m, ArH), 7.45 (2H, m, ArH), 7.34 (2H, m, ArH), 6.15 (1H, m, NH), 4.73 (1H, m, CH), 4.60 (1H, m, Fmoc–CH_2), 4.39 (1H, m, Fmoc–CH_2), 4.30 (1H, t, J=7.0 Hz, Fmoc–CH), 3.05 (1H, dd, J=17.0, 4.5 Hz, CH_2CO), 2.92 (2H, q, J=7.5 Hz, SCH_2CH$_3$), 2.75 (1H, dd, J=17.0, 4.5 Hz, CH_2CO), 1.49 (9H, s, *t*Bu), 1.29 (3H, t, J=7.0 Hz, SCH$_2$$CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 200.3, 170.1, 155.94, 143.9, 143.7, 141.3, 127.8, 127.1, 125.2, 125.1, 120.0, 82.0, 67.4, 57.4, 47.2, 37.5, 28.0, 23.6, 23.6, 14.4; HRMS (ESI) $C_{25}H_{29}NNaO_5S$ calcd for $(MNa)^+$: 478.1661, found: 478.1636.

3.2.2. Ethyl *N*-Fmoc-thionorleucinate. 88% yield as white solid; R_f=0.78 (SiO_2, EtOAc–hexanes 1:1); IR (KBr): 3305.5, 2951.9, 1694.1, 1535.6, 1450.4 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.80 (2H, m, ArH), 7.52 (2H, m, ArH), 7.44 (2H, m, ArH), 7.35 (2H, m, ArH), 5.26 (1H, d, J=9.0 Hz, NH), 4.53 (1H, m, CH), 4.42 (2H, m, Fmoc–CH_2), 4.28 (1H, t, J=7.0 Hz, Fmoc–CH), 2.92 (2H, q, J=8.0 Hz, SCH_2CH$_3$), 1.92 (1H, m, CH_2CH$_2$CH$_2$CH$_3$), 1.65 (1H, m, CH_2CH$_2$CH$_2$CH$_3$), 1.37 (4H, m, CH$_2$$CH_2$$CH_2CH_3$), 1.29 (3H, t, J=8.0 Hz, SCH$_2$$CH_3$), 0.94 (3H, m, CH$_2CH_2$-CH$_2$$CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 201.0, 155.8, 143.9, 143.7, 141.3, 127.7, 127.1, 125.1, 125.0, 120.0, 67.0, 61.0, 47.2, 32.6, 27.3, 23.28, 22.2, 14.5, 13.8; HRMS (ESI) calcd for $C_{23}H_{27}NNaO_3S$ $(MNa)^+$: 420.1606, found: 420.1607.

3.2.3. Ethyl *N*-Fmoc-*N*-γ-trityl-thioglutaminate. 95% yield as white solid; R_f=0.55 (SiO_2, EtOAc–hexanes 1:1); IR (KBr): 3310.5, 3056.5, 2928.3, 1679.1, 1492.4, 1447.2 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79 (2H, d, J=7.5 Hz, ArH), 7.64 (2H, m, ArH), 7.42 (2H, m, ArH), 7.32 (2H, m, ArH), 6.85 (1H, s, NH), 7.25 (2H, m, Ar–H), 5.88 (1H, d, J=7.0 Hz, NH), 4.56 (1H, m, CH), 4.37 (2H, m,

Fmoc–CH_2), 4.27 (1H, t, J=7.0 Hz, Fmoc–CH), 2.91 (2H, q, J=7.0 Hz, S$CH_2$$CH_3$), 2.42 (2H, m, $CH_2$$CH_2$CO), 2.23 (1H, m, $CH_2$$CH_2$CO), 2.04 (1H, m, $CH_2$$CH_2$CO), 1.27 (3H, t, J=7.0 Hz, SCH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 201.0, 171.0, 156.2, 144.5, 143.9, 143.7, 141.3, 128.7, 128.0, 127.8, 127.7, 125.3, 125.2, 120.0, 70.7, 67.1, 47.3, 33.3, 27.9, 23.3, 14.4; HRMS (ESI) calcd for $C_{41}H_{38}N_2NaO_4S$ $(MNa)^+$: 677.2448, found: 677.2483.

3.2.4. Ethyl *N*-Fmoc-*N*-ε-Boc-thiolysinate. 94% yield as white solid; R_f=0.51 (SiO_2, EtOAc–hexanes 1:1); IR (KBr): 3352.2, 2930.8, 1688.3, 1526.2, 1449.1 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79 (2H, d, J=8.0 Hz, ArH), 7.66 (2H, m, ArH), 7.43 (2H, m, ArH), 7.34 (2H, m, ArH), 5.52 (1H, m, NH), 4.62 (1H, s, NH), 4.55 (1H, m, CH), 4.41 (2H, m, Fmoc–CH_2), 4.27 (1H, m, Fmoc–CH), 3.14 (2H, m, $CH_2CH_2CH_2$$CH_2$NH), 2.92 (2H, q, J=7.5 Hz, S$CH_2$$CH_3$), 1.92 (1H, m, $CH_2$$CH_2CH_2CH_2$NH), 1.70 (1H, m, $CH_2$$CH_2$-$CH_2CH_2$NH), 1.47 (13H, m, *t*Bu and $CH_2$$CH_2$$CH_2CH_2$NH), 1.28 (3H, t, J=7.5 Hz, SCH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 201.00, 156.2, 156.0, 143.9, 143.7, 141.3, 127.7, 127.1, 125.1, 120.0, 79.22, 67.1, 60.8, 47.2, 39.8, 32.2, 29.7, 28.4, 23.3, 22.3, 14.5; HRMS (ESI) $C_{23}H_{27}NNaO_3S$ calcd for $(MNa)^+$: 535.2240, found: 535.2222.

3.2.5. Aldehyde 8; general procedure for reduction of thioester. To a stirring solution of the thioester (vide supra) (0.2–0.3 M) and of Pd/C 5% (2 mol%) in CH_2Cl_2 was added Et_3SiH (4.0 equiv.). The reaction mixture was stirred at 23 °C until TLC analysis revealed complete consumption of the starting material (30–40 min). The crude reaction mixture was loaded directly onto a silica gel column and eluted with EtOAc–hexanes.

*N-Fmoc-O-tButyl-aspartal (**8-Asp**)*. 87% yield as viscous oil; R_f=0.56 (SiO_2, EtOAc–hexanes 1:1); IR (KBr): 3355.9, 2977.8, 1724.4, 1515.7, 1450.0 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.67 (1H, s, HCO), 7.68 (2H, d, J=7.5 Hz, ArH), 7.63 (2H, d, J=7.5 Hz, ArH), 7.44 (2H, m, ArH), 7.35 (2H, m, ArH), 5.99 (1H, d, J=7.0 Hz, NH), 4.46 (3H, m, Fmoc–CH_2 and CH), 4.30 (1H, t, J=7.0 Hz, Fmoc–CH), 2.97 (1H, dd, J=17.0, 4.5 Hz, CH_2CO), 2.83 (1H, dd, J=17.0, 4.5 Hz, CH_2CO), 1.49 (9H, s, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 198.8, 170.2, 156.2, 143.8, 143.7, 141.3, 127.8, 127.1, 125.1, 120.0, 82.2, 67.3, 56.6, 47.1, 35.6, 28.0; HRMS (ESI) $C_{23}H_{25}NNaO_5$ calcd for $(MNa)^+$: 418.1625, found: 418.1576.

N-Fmoc-norleucinal (**8-Nle**). 77% yield as white solid; R_f=0.73 (SiO_2, EtOAc–hexanes 1:1); IR (KBr): 3321.7, 2955.0, 1738.3, 1689.0, 1537.0, 1448.9 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.62 (1H, s, HCO), 7.80 (2H, m, ArH), 7.64 (2H, m, ArH), 7.44 (2H, m, ArH), 7.35 (2H, m, ArH), 5.34 (1H, d, J=9.0 Hz, NH), 4.47 (2H, d, J=7.0 Hz, Fmoc–CH_2), 4.35 (1H, m, CH), 4.26 (1H, t, J=7.0 Hz, Fmoc–CH), 1.95 (1H, m, $CH_2CH_2CH_2CH_3$), 1.66 (1H, m, $CH_2CH_2CH_2CH_3$), 1.38 (4H, m, $CH_2$$CH_2$$CH_2CH_3$), 0.95 (3H, m, $CH_2CH_2CH_2$$CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 199.3, 143.73, 141.3, 127.8, 127.1, 125.0, 120.0, 66.9, 60.2, 47.2, 28.9, 27.1, 22.5, 13.8; HRMS (ESI) calcd for $C_{21}H_{23}NNaO_3$ $(MNa)^+$: 360.1570, found: 360.1592.

N-α-Fmoc-N-γ-trityl-glutaminal (**8-Gln**). 94% yield as white solid; R_f=0.35 (SiO_2, EtOAc–hexanes 1:1); IR (KBr): 3316.9, 3057.4, 1714.0, 1671.8, 1492.5, 1447.6 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.47 (1H, s, HCO), 7.70 (2H, m, ArH), 7.62 (2H, m, ArH), 7.40 (2H, m, ArH), 7.30 (15H, m, ArH), 7.23 (2H, m, ArH), 6.83 (1H, s, NH), 5.61 (1H, d, J=6.0 Hz, NH), 4.49 (1H, m, CH), 4.16 (2H, q, J=7.0 Hz, Fmoc–CH_2), 4.24 (1H, t, J=7.0 Hz, Fmoc–CH), 2.35 (2H, m, $CH_2$$CH_2$CO), 2.26 (1H, m, CH_2CH_2CO), 1.86 (1H, m, CH_2CH_2CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 198.6, 170.9, 156.4, 144.5, 143.7, 141.4, 128.6, 128.0, 127.7, 127.1, 125.0, 120.0, 70.7, 66.7, 59.4, 47.3, 32.5, 24.6; HRMS (ESI) calcd for $C_{39}H_{34}N_2NaO_4$ $(MNa)^+$: 617.2411, found: 617.2428.

N-α-Fmoc-N-ε-Boc-lysinal (**8-Lys**). 80% yield as white solid; R_f=0.40 (SiO_2, EtOAc–hexanes 1:1); IR (KBr): 3352.3, 2929.8, 1686.2, 1525.0, 1449.9 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.60 (1H, s, HCO), 7.79 (2H, d, J=7.5 Hz, ArH), 7.64 (2H, d, J=7.5 Hz, ArH), 7.44 (2H, m, ArH), 7.35 (2H, m, ArH), 5.54 (1H, s, NH), 4.62 (1H, s, NH), 4.46 (2H, m, Fmoc–CH_2), 4.31 (1H, m, CH), 4.25 (1H, t, J=7.0 Hz, Fmoc–CH), 3.15 (2H, m, $CH_2CH_2CH_2$$CH_2$NH), 1.96 (1H, m, $CH_2$$CH_2CH_2CH_2$NH), 1.70 (1H, m, $CH_2CH_2CH_2CH_2$NH), 1.46 (13H, m, *t*Bu and $CH_2$$CH_2$$CH_2CH_2$NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 199.4, 156.2, 143.7, 141.3, 127.1, 125.0, 120.0, 79.3, 67.0, 60.0, 47.2, 40.0, 29.8, 28.5, 28.4, 22.1; HRMS (ESI) $C_{26}H_{32}N_2NaO_5$ calcd for $(MNa)^+$: 475.2204, found: 475.2210.

3.2.6. Allyl acrylic ester derivatives; general procedure for the Wittig olefination. To a stirring solution of the aldehyde **8** (0.1 M) and allylphosphonium iodide (1.6 equiv.) in toluene at 80 °C was added Et*i*Pr_2N (1.4 equiv.). The reaction mixture was stirred at 80 °C until TLC analysis revealed complete consumption of the starting material (90–120 min). The reaction mixture was diluted in EtOAc and washed with 0.1 N HCl, sat. $NaCO_3$, brine, dried over $MgSO_4$ and purified by silica gel chromatography (EtOAc–hexanes).

Allyl N-Fmoc-O-tButyl-acrylicaspartate. 83% yield as viscous oil; R_f=0.86 (SiO_2, $CHCl_3$–MeOH 9:1); IR (KBr): 3336.7, 2978.5, 1725.1, 1526.0 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79 (2H, d, J=7.5 Hz, ArH), 7.62 (2H, d, J=7.5 Hz, ArH), 7.43 (2H, m, ArH), 7.34 (2H, m, ArH), 6.97 (1H, dd, J=16.0, 5.0 Hz, CH=CHCO), 5.98 (2H, m, CH_2=$CH$$CH_2$O and CH=$CH$CO), 5.70 (1H, d, J=8.5 Hz, NH), 5.38 (1H, dd, J=17.0, 1.5 Hz, CH_2=CHCH_2-O), 5.28 (1H, dd, J=10.5, 1.5 Hz, CH_2=$CHCH_2$O), 4.75 (1H, m, CH), 4.68 (2H, d, J=6.0 Hz, CH_2=CHCH_2O), 4.45 (2H, m, Fmoc–CH_2), 4.25 (1H, t, J=7.0 Hz, Fmoc–CH), 2.68 (1H, dd, J=16.0, 5.0 Hz, CH_2CO), 2.59 (1H, dd, J=16.0, 5.0 Hz, CH_2CO), 1.47 (9H, s, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.8, 165.5, 155.5, 146.6, 143.8, 141.3, 129.4, 128.0, 127.5, 125.6, 120.5, 118.4, 82.0, 67.0, 65.2, 48.8, 47.2, 39.4, 28.0; HRMS (ESI) $C_{28}H_{31}NNaO_6$ calcd for $(MNa)^+$: 500.2044, found: 500.2040.

Allyl N-Fmoc-acrylicnorleucinate. 86% yield as white solid; R_f=0.73 (SiO_2, $CHCl_3$–MeOH 9:1); IR (KBr): 3303.3, 2954.2, 1727.8, 1689.7, 1540.6, 1449.8 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.80 (2H, d, J=7.5 Hz, ArH), 7.63 (2H, d, J=7.5 Hz, ArH), 7.43 (2H, m, ArH), 7.35 (2H,

m, ArH), 6.91 (1H, dd, *J*=15.5, 5.2 Hz, *CH*═CHCO), 5.98 (2H, m, CH_2═*CH*CH_2O and CH═*CH*CO), 5.38 (1H, dd, *J*=17.0, 1.5 Hz, *CH*$_2$═CHCH_2O), 5.29 (1H, dd, *J*=10.5, 1.5 Hz, *CH*$_2$═CHCH_2O), 4.85 (1H, d, *J*=8.0 Hz, NH), 4.68 (2H, d, *J*=6.0 Hz, CH_2═CH*CH*$_2$O), 4.48 (2H, d, *J*=7.0 Hz, Fmoc–CH_2), 4.37 (1H, m, CH), 4.25 (1H, t, *J*=7.0 Hz, Fmoc–CH), 1.63 (1H, m, *CH*$_2$$CH_2CH_2CH_3$), 1.55 (1H, m, *CH*$_2$$CH_2CH_2CH_3$), 1.35 (4H, m, CH_2*CH*$_2$*CH*$_2$$CH_3$), 0.93 (3H, m, $CH_2CH_2CH_2$*CH*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 165.9, 155.7, 148.6, 143.3, 141.3, 132.1, 127.7, 127.1, 125.0, 120.6, 120.0, 118.4, 66.6, 65.2, 52.0, 47.3, 34.2, 27.7, 22.3, 13.9; HRMS (ESI) calcd for $C_{26}H_{29}NNaO_4$ $(MNa)^+$: 442.1989, found: 442.2021.

Allyl N-α-Fmoc-N-γ-trityl-acrylicglutamate. 90% yield as white solid; R_f=0.85 (SiO_2, $CHCl_3$–MeOH 9:1); IR (KBr): 3314.3, 3058.5, 2928.5, 1718.5, 1665.8, 1517.1, 1492.3, 1447.0 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.78 (2H, d, *J*=8.0 Hz, ArH), 7.60 (2H, m, ArH), 7.41 (2H, m, ArH), 7.23–7.43 (17H, m, ArH), 6.86 (1H, dd, *J*=16.0, 5.0 Hz, *CH*═CHCO), 6.80 (1H, s, NH), 5.96 (2H, m, CH_2═*CH*CH_2O and CH═*CH*CO), 5.27–5.39 (3H, m, *CH*$_2$═CHCH_2O and NH), 4.67 (2H, d, *J*=6.0 Hz, CH_2═CH*CH*$_2$O), 4.49 (1H, m, CH), 4.41–4.54 (2H, m, Fmoc–CH_2), 4.22 (1H, t, *J*=7.0 Hz, Fmoc–CH), 2.35 (2H, m, CH_2*CH*$_2$CO), 1.97 (1H, m, *CH*$_2$$CH_2CO$), 1.82 (1H, m, *CH*$_2$$CH_2CO$); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 171.1, 165.7, 156.0, 147.7, 144.5, 143.3, 141.3, 132.1, 128.7, 128.0, 127.7, 127.1, 125.1, 121.1, 119.98, 118.4, 70.7, 66.6, 65.3, 51.7, 47.32, 33.4, 29.2; HRMS (ESI) calcd for $C_{44}H_{40}N_2NaO_5(MNa)^+$: 699.2830, found: 699.2857.

Allyl N-α-Fmoc-N-ε-Boc-acryliclysinate. 83% yield as white solid; R_f=0.70 (SiO_2, $CHCl_3$–MeOH 9:1); IR (KBr): 3347.6, 2937.0, 1716.6, 1692.8, 1528.1, 1450.3 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.80 (2H, d, *J*=7.0 Hz, ArH), 7.62 (2H, d, *J*=7.0 Hz, ArH), 7.44 (2H, m, ArH), 7.35 (2H, m, ArH), 5.97 (2H, m, CH_2═*CH*CH_2O and CH═*CH*CO), 6.86 (1H, dd, *J*=10.5, 1.5 Hz, *CH*═CHCO), 5.37 (1H, dd, *J*=17.0, 1.5 Hz, *CH*═CHCO), 4.89 (1H, s, NH), 4.67 (2H, d, *J*=6.0 Hz, CH_2═CH*CH*$_2$O), 4.48 (2H, m, Fmoc–CH_2), 4.57 (1H, s, NH), 4.36 (1H, m, CH), 4.24 (1H, t, *J*=7.0 Hz, Fmoc–CH), 3.15 (2H, m, $CH_2CH_2CH_2$*CH*$_2$NH), 1.60 (2H, m, *CH*$_2$$CH_2CH_2CH_2$NH), 1.46 (13H, m, *t*Bu and CH_2*CH*$_2$*CH*$_2$$CH_2$NH); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 165.9, 156.2, 155.9, 148.5, 143.9, 143.8, 141.3, 132.1, 127.7, 127.1, 125.0, 124.8, 120.6, 120.0, 118.4, 79.1, 66.6, 65.2, 52.0, 47.2, 40.0, 33.8, 29.7, 28.4, 22.8; HRMS (ESI) $C_{31}H_{38}N_2NaO_6$ calcd for $(MNa)^+$: 557.2622, found: 557.2649.

3.2.7. Acrylic acid 10; general procedure for the allyl ester deprotections. To a stirring solution of the allyl ester (0.1 M) and $Pd(PPh_3)_4$ (0.1 equiv.) in CH_2Cl_2 was added *n*Bu_3SnH (5.0 equiv.). The reaction mixture was stirred at 23 °C 90 min. The crude reaction mixture loaded directly onto a silica gel column and eluted with MeOH–CH_2Cl_2.

N-Fmoc-O-tButyl-acrylicaspartic acid (**10-Asp**). 45% yield as white-yellow solid; R_f=0.31 (SiO_2, $CHCl_3$–MeOH 9:1); IR (KBr): 3398.5, 2976.6, 1721.4, 1542.9, 1411.3 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 7.89 (2H, d, *J*=7.0 Hz, ArH), 7.70 (2H, m, ArH), 7.62 (1H, d, *J*=8.5 Hz, NH), 7.41 (2H, m, ArH), 7.33 (2H, m, ArH), 6.51 (1H, dd, *J*=15.5, 5.5 Hz, *CH*═CHCO$_2$H), 5.77 (1H, d, *J*=15.5 Hz, CH═*CH*CO$_2$H), 4.48 (1H, m, CH), 4.30 (2H, m, Fmoc–CH_2), 4.23 (1H, t, *J*=7.0 Hz, Fmoc–CH), 2.48 (2H, m, CH_2CO), 1.37 (9H, s, *t*Bu); ^{13}C NMR (100 MHz, DMSO-d_6) *δ*: 169.8, 155.8, 144.28, 141.2, 129.4, 128.1, 127.5, 125.6, 121.8, 120.6, 120.5, 80.5, 66.0, 49.3, 47.1, 40.5, 28.1; HRMS (ESI) $C_{25}H_{27}NNaO_6$ calcd for $(MNa)^+$: 460.1731, found: 460.1730.

N-Fmoc-acrylicnorleucine (**10-Nle**). 86% yield as white solid; R_f=0.43 (SiO_2, $CHCl_3$–MeOH 9:1); IR (KBr): 3401.2, 2954.8, 1701.7, 1542.6, 1409.4 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 7.89 (2H, d, *J*=7.0 Hz, ArH), 7.72 (2H, d, *J*=7.0 Hz, ArH), 7.51 (d, *J*=8.5 Hz, 1H, NH), 7.41 (2H, m, ArH), 7.33 (2H, m, ArH), 6.53 (1H, dd, *J*=15.0, 6.0 Hz, *CH*═CHCO$_2$H), 5.76 (1H, d, *J*=15.0 Hz, CH═*CH*CO$_2$H), 4.30 (2H, m, Fmoc–CH_2), 4.23 (1H, m, Fmoc–CH), 4.06 (1H, m, CH), 1.48 (2H, m, *CH*$_2$$CH_2CH_2CH_3$), 1.27 (4H, s, CH_2*CH*$_2$*CH*$_2$$CH_3$), 0.86 (3H, s, $CH_2CH_2CH_2$*CH*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 156.1, 144.4, 144.3, 141.2, 141.1, 128.1, 127.5, 125.7, 125.6, 120.5, 65.8, 52.0, 47.2, 34.1, 28.2, 22.3, 14.5; HRMS (ESI) calcd for $C_{23}H_{25}NNaO_4$ $(MNa)^+$: 402.1676, found: 402.1679.

N-α-Fmoc-N-γ-trityl-acrylicglutamine acid (**10-Gln**). 89% yield as white-yellow solid; R_f=0.40 (SiO_2, $CHCl_3$–MeOH 9:1); IR (KBr): 3405.8, 3057.5, 1698.8, 1494.1, 1409.2 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 8.65 (1H, s, CO_2H), 7.95 (2H, m, ArH), 7.73 (2H, m, ArH), 7.56 (1H, d, *J*=8.5 Hz, NH), 7.42 (2H, m, ArH), 7.34 (2H, m, ArH), 7.23 (6H, m, ArH), 7.19 (9H, m, ArH), 6.51 (1H, dd, *J*=16.0, 6.0 Hz, *CH*═CHCO$_2$H), 5.77 (1H, d, *J*=16.0 Hz, CH═*CH*CO$_2$H), 4.23–4.32 (3H, m, Fmoc–CH and Fmoc–CH_2), 4.10 (1H, m, CH), 2.33 (2H m, CH_2*CH*$_2$CO), 1.68 (2H, m, *CH*$_2$$CH_2CO$); ^{13}C NMR (100 MHz, DMSO-d_6) *δ*: 171.9, 145.4, 144.4, 144.3, 141.2, 141.1, 129.4, 129.0, 128.0, 127.9, 126.7, 125.7, 121.8, 120.6, 110.2, 69.6, 65.9, 51.8, 47.2, 33.2, 30.5; HRMS (ESI) calcd for $C_{41}H_{36}N_2NaO_5$ $(MNa)^+$: 659.2517, found: 659.2567.

N-α-Fmoc-N-ε-Boc-acryliclysine (**10-Lys**). 91% yield as white solid; R_f=0.23 (SiO_2, $CHCl_3$–MeOH 9:1); IR (KBr): 3338.4, 2934.2, 1701.5, 1526.2, 1409.8 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 7.89 (2H, d, *J*=7.5 Hz, ArH), 7.72 (2H, d, *J*=7.5 Hz, ArH), 7.51 (1H, d, *J*=8.5 Hz, NH), 7.42 (2H, m, ArH), 7.34 (2H, m, ArH), 6.78 (1H, m, NH), 6.55 (1H, dd, *J*=15.5, 6.0 Hz, *CH*═CHCO$_2$H), 5.77 (1H, d, *J*=15.5 Hz, CH═*CH*CO$_2$H), 4.30 (2H, m, Fmoc–CH_2), 4.23 (1H, m, Fmoc–CH), 4.06 (1H, m, CH), 2.90 (2H, m, $CH_2CH_2CH_2$*CH*$_2$NH), 1.48 (2H, m, *CH*$_2$$CH_2CH_2CH_2$NH), 1.37 (9H, m, *t*Bu), 1.27 (4H, m, CH_2*CH*$_2$*CH*$_2$$CH_2$NH); ^{13}C NMR (100 MHz, DMSO-d_6) *δ*: 156.1, 156.0, 144.34, 144.3, 141.2, 129.4, 128.1, 127.5, 125.7, 125.6, 121.8, 120.5, 77.8, 65.8, 52.1, 47.2, 40.2, 34.0, 29.7, 28.7, 23.4; HRMS (ESI) $C_{28}H_{34}N_2NaO_6$ calcd for $(MNa)^+$: 517.2309, found: 517.2284.

3.3. Alloc-protected PNA monomers 14

The reactions were carried out in parallel on an Argonaut Quest according to the following procedure. 2-Chlorotritylchloride resin (75–100 mesh, 1% DVB, 1.6 mmol/g,

2.0 equiv.) preswelled in CH_2Cl_2 was treated with a solution of acid **11** (1.0 equiv.) and Et*i*Pr$_2$N (1.0 equiv.) in CH_2Cl_2 (10 mL/g). After 3 h, the resin was filtered and washed (CH_2Cl_2), and the resins were treated with DBU (1.0 equiv.) in CH_2Cl_2 (10 mL/g) for 5 min to induce Fmoc deprotection. After washing (CH_2Cl_2), the resins were suspended in CH_2Cl_2, cooled to 0 °C and Et*i*Pr$_2$N (4.0 equiv.) and allyl chloroformate (3.0 equiv.) in CH_2Cl_2 as a 1 M CH_2Cl_2 solution were added. The reaction was carried out twice for 10 min at 0 °C then washed and cleaved with AcOH–CF_3CH_2OH–CH_2Cl_2 (1:1:8) (5 mL/g of resin) for 1 h to obtain **14** which were precipitated in diethyl ether, pelletted by centrifugation (9000 g), resuspended in 1:1 MeCN–H_2O and lyophilized.

3.3.1. *N*-(2-Allyloxyaminoethyl)-*N*-(thymine-1-acetyl)-glycine (14-T). 60% yield as viscous oil; IR (KBr): 3422.0, 3054.7, 1707.7, 1540.9, 1474.2 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.32 (1H, m, C_6), 5.91 (1H, m, CH_2=*CH*CH_2O), 5.27 (1H, dd, J=17.0, 1.5 Hz, *CH_2*=$CHCH_2O$), 5.18 (1H, m, *CH_2*=$CHCH_2O$), 4.66 (1H, CH_2=CH*CH_2*O), 4.46–4.50 (3H, m, CH_2=CH*CH_2*O and CH_2CO), 4.08 (0.6H, s, *CH_2CO_2H*), 3.98 (1.4H, s, *CH_2CO_2H*), 3.44–3.10 (4H, m, CH_2CH_2), 1.17 (3H, s, Me); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.9, 167.6, 164.8, 156.4, 151.4, 142.5, 134.1, 117.5, 108.53, 64.9, 48.0, 47.5, 47.1, 44.6, 12.3; HRMS (ESI) calcd for $C_{15}H_{20}NaN_4O_7$ (MNa^+): 391.1224, found: 391.1187.

3.3.2. *N*-(2-Allyloxyaminoethyl)-*N*-[4-*N*-(benzhydryloxy-carbonyl)cytosine-1-acetyl]glycine (14-C). 46% yield as viscous oil; IR (KBr): 3404.5, 3066.7. 2947.0, 1719.7, 1561.6, 1499.9 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: rotamer (7.90, 7.87) (d, 1H, J=7.5 Hz, C_6), 7.45 (4H, m, ArH), 7.38 (4H, m, ArH), 7.30 (2H, m, ArH), 6.94 (1H, m, C_5), 6.81 (1H, s, *$CH(C_6H_5)_2$*), 5.91 (1H, m, CH_2=*CH*CH_2O), (5.27, 5.16) (2H, m, *CH_2*=$CHCH_2O$), (4.82, 4.62) (2H, s, CH_2CON), (4.50, 4.46) (2H, d, J=5.5 Hz, CH_2=CH*CH_2*O), (4.12, 3.99) (2H, s, N*CH_2CO_2H*), 3.11–3.47 (4H, m, NH*CH_2CH_2*N); ^{13}C NMR (100 MHz, DMSO-d_6) δ: (171.4, 171.0), (168.0, 167.4), 163.5, 155.4, 152.8, 151.4, 151.4, 142.3, 140.8, 134.1, 128.0, 128.3, 126.9, 117.5, (94.2, 93.1), 77.9, 64.9, 64.7, 49.9, 48.2, 47.3, 43.4; HRMS (ESI) calcd for $C_{28}H_{29}N_5O_8$ (MH^+): 564.2089, found: 564.211.

3.3.3. *N*-(2-Allyloxyaminoethyl)-*N*-[6-*N*-(benzhydryloxy-carbonyl)adenine-9-acetyl]glycine (14-A). 58% yield as viscous oil; IR (KBr): 3419.4, 3068.7, 2935.0, 1718.6, 1522.2, 1467.1 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.60 (1H, s, C_2), 8.34 (1H, s, C_8), 7.54 (4H, d, J=7.5 Hz, ArH); 7.39 (4H, t, J=7.5 Hz, ArH), 7.30 (2H, t, J=7.5 Hz, ArH), 6.83 (1H, s, O*$CH(C_6H_5)_2$*), 5.91 (1H, m, CH_2=*CH*CH_2O), rotamer (5.36, 5.17) (s, 2H, N*CH_2*CON), (5.32, 5.27), (2H, m, *CH_2*=$CHCH_2O$), (4.53, 4.46) (2H, d, J=5.5 Hz, CH*CH_2*O), (4.33, 4.02) (2H, s, N*CH_2CO_2H*), 3.59–3.11 (4H, m, NH*CH_2CH_2*N); ^{13}C NMR (100 MHz, DMSO-d_6) δ: (171.9, 170.8), (167.5, 167.0), (156.7, 156.4), 152.7, (151.9, 151.6), 149.7, (145.7, 145.6), 141.3, 134.1, 128.9, 128.1, 126.9, 123.0, 117.5, 117.4, 77.7, (65.0, 64.7), 49.8, (48.1, 47.3), (44.5, 44.3); HRMS (ESI) calcd for $C_{28}H_{30}N_7O_5$: ($M-CO_2+H^+$): 544.2303, found: 544.2254.

3.3.4. *N*-(2-Allyloxyaminoethyl)-*N*-[2-*N*-(benzhydryloxy-carbonyl)-guanine-9-acetyl]glycine (14-G). 61% yield as viscous oil; IR (KBr): 3377.8, 3246.0, 3066.7, 2944.9, 1731.6, 1486.3 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ rotamer (8.35, 8.33, 7.83, 7.81) (1H, s, C_8), 7.46 (4H, d, J=7.0 Hz, ArH); 7.37 (4H, t, J=7.5 Hz, ArH), 7.31 (2H, t, J=7.5 Hz, ArH), (6.88, 6.87, 6.80, 6.79) (1H, s, O*$CH(C_6H_5)_2$*), (6.01, 5.88) (1H, m, OCH_2*CH*=CH_2), (5.48- 5.42) (1H, m, NH), (5.36, 5.33, 5.30, 5.26) (1H, s, N*H*CH_2CH_2N), (5.27, 5.23, 5.18, 5.16) (1H, d, J=15.5 Hz, *CH_2*=$CHCH_2O$), (5.13, 4.21) (2H, m, N*CH_2*CON), (4.51, 4.46) (2H, m, CH_2=CH*CH_2*O), (4.84–4.45) (2H, m, CH*CH_2*CO), (4.30, 4.01) (2H, s, N*CH_2CO_2H*), 3.54–3.12 (4H, m, NH*CH_2CH_2*N); ^{13}C NMR (100 MHz, DMSO-d_6) δ: (171.3, 170.9), (167.5, 166.9), 156.7, 155.5, (154.2, 154.1), 149.8, 147.5, (140.9, 140.4), 134.0, (129.0, 128.9), 128.4, 126.9, (120.2, 120.0), 119.6, (117.6, 117.4), 78.5, (65.0, 64.7), 48.3, 47.3, 44.3; HRMS (ESI) calcd for $C_{28}H_{29}N_7O_6$: ($M-CO_2+H^+$): 560.2252, found: 560.2206.

3.4. Alloc-protected amino acids 25

The reactions were carried out in parallel on an Argonaut Quest according to the following procedure. To 2-Chlorotritylchloride resin (75–100 mesh, 1% DVB, 1.6 mmol/g, 1.0 equiv.) swelled in CH_2Cl_2 (5 mL/g) was added a solution of acid (1.4 equiv.) and Et*i*Pr$_2$N (2.0 equiv.) in CH_2Cl_2 (5 mL/g of resin). After 3 h, the resin was filtered and washed (CH_2Cl_2) and the Fmoc was removed by standard piperidine treatment (20% in DMF, 1.5 mL, 10 min). After washing, the resins were suspended in CH_2Cl_2 (5 mL/g), cooled to 0 °C and treated sequentially with Et*i*Pr$_2$N (4.0 equiv.) followed by allyl chloroformate (3.0 equiv.) as 1M solution in CH_2Cl_2. The reaction was repeated once for 10 min before washing and cleaving with TFA (2.5% in CH_2Cl_2, 10 mL/g) during 15 min. The filtrates were diluted with toluene (1:1 v:v), concentrated then lyophilised from AcCN–H_2O.

3.4.1. Alloc-Ala-OH. 72% yield as viscous oil; IR (KBr): 3328.5, 2994.9, 1714.0, 1537.7, 1455.8 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.69 (1H, bs, CO_2H), 6.77 (1H, s, NH), 5.93 (1H, ddt, J=17.0, 10.5, 5.0 Hz), 5.34 (1H, dd, J=17.0, 1.5 Hz, *CH_2*=$CHCH_2O$), 5.25 (1H, d, J=10.5 Hz, *CH_2*=$CHCH_2O$), 4.61 (1H, d, J=5.0 Hz, CH_2=CH*CH_2*O), 4.43 (1H, m, CH), 1.49 (3H, d, J=7.0 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 177.5, 155.8, 132.4, 118.0, 66.5, 49.4, 18.3; HRMS (ESI) calcd for $C_7H_{11}NaNO_4$ $(MNa)^+$: 196.0580, found: 196.0588.

3.4.2. Alloc-Arg(Pfb)-OH. 54% yield as viscous oil; IR (KBr): 3341.9, 2982.9, 1670.1, 1576.1, 1457.7; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.88 (1H, bs, CO_2H), 7.60 (1H, s, NH), 6.50 (1H, s, NH), 6.16 (1H, s, NH), 5.87 (1H, m, CH_2=*CH*CH_2O), 5.29 (1H, d, J=17.0 Hz, *CH_2*=$CHCH_2O$), 5.20 (1H, d, J=10.0 Hz, *CH_2*=$CHCH_2O$), 4.54 (2H, s, CH_2=CH*CH_2*O), 4.28 (1H, s, CH), 3.32 (2H, m, CH_2CH_2*CH_2*NH), 3.00 (2H, s, *$CH_2C(CH_3)_2$*), 2.51 (3H, s, ArMe), 2.47 (3H, s, ArMe), 2.12 (3H, s, ArMe), 1.91–1.73 (4H, s, *$CH_2CH_2CH_2$*NH), 1.50 (6H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.9, 169.2, 162.7, 156.9, 137.9, 134.7, 132.3, 129.0, 128.2, 125.3, 117.8, 87.6, 66.2, 53.2, 42.9, 41.3, 29.2, 28.5, 24.0, 19.2, 12.4; HRMS (ESI) calcd for $C_{23}H_{36}N_4O_7S$ $(MH)^+$: 511.2221, found: 511.2192.

3.4.3. Alloc-Asp(*t*Bu)-OH. 84% yield as viscous oil; IR (KBr): 3341.9, 2982.0, 1728.0, 1519.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.87 (1H, bs, CO_2H), 5.91 (1H, ddt, J=17.0, 10.0, 5.0 Hz, CH_2=$CH$$CH_2O$), 5.84 (1H, d, J=8.0 Hz, NH), 5.33 (1H, dd, J=17.0, 1.5 Hz, CH_2=$CHCH_2O$), 5.23 (1H, dd, J=10.0, 1.5 Hz, CH_2=$CHCH_2O$), 4.61 (3H, m, CH_2=$CHCH_2O$ and CH), 2.98 (1H, dd, J=17.0, 5.0 Hz, CH_2O*t*Bu), 2.79 (1H, dd, J=17.0, 5.0 Hz, CH_2O*t*Bu), 1.45 (9H, s, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 181.8, 176.3, 162.2, 138.5, 124.1, 88.4, 72.3, 56.5, 43.8, 34.1; HRMS (ESI) calcd for $C_{12}H_{19}NaNO_6$ $(MNa)^+$: 296.11045, found: 296.1119.

3.4.4. Alloc-His(Boc)-OH. 85% yield; IR (KBr): 3329.9, 2935.0, 1720.1, 1528.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.18 (1H, s, CHNCHN), 7.25 (1H, s, CHNCHN), 5.95 (1H, m, CH_2=$CH$$CH_2O$), 5.86 (1H, d, J=10.5 Hz, NH), 5.35 (1H, d, J=17.0 Hz, CH_2=$CHCH_2O$), 5.25 (1H, d, J=10.5 Hz, CH_2=$CHCH_2O$), 4.61 (2H, d, J=5.5 Hz, CH_2=$CHCH_2O$), 4.56 (1H, m, CH), 3.14 (2H, m, $CHCH_2C$), 1.63 (9H, s, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$): 173.0, 162.3, 155.7, 146.2, 136.8, 132.7, 117.8, 115.5, 86.6, 65.7, 53.2, 29.7, 27.8; HRMS (ESI) calcd for $C_{15}H_{22}N_3O_6$ $(MH)^+$: 340.1503, found: 340.1513

3.4.5. Alloc-Lys(Boc)-OH. 91% yield as viscous oil; IR (KBr): 3346.0, 2935.0, 1701.0, 1522.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.36 (1H, s, NH), 5.91 (1H, m, CH_2=$CH$$CH_2O$), 5.73 (1H, d, J=8.0 Hz, NH), 5.31 (1H, d, J=17.0 Hz, CH_2=$CHCH_2O$), 5.22 (1H, d, J=10.0 Hz, CH_2=$CHCH_2O$), 4.58 (2H, d, J=5.0 Hz, CH_2=$CHCH_2O$), 4.37 (1H, m, CH), 3.11 (2H, m, $CH_2CH_2CH_2CH_2NH$), 1.88 (1H, m, $CH_2CH_2CH_2CH_2NH$), 1.76 (1H, m, $CH_2CH_2CH_2CH_2NH$), 1.45 (13H, s, *t*Bu and $CH_2CH_2CH_2CH_2NH$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.9, 175.5, 156.2, 132.6, 117.8, 79.6, 65.9, 53.6, 40.0, 31.8, 29.5, 28.4; HRMS (ESI) calcd for $C_{15}H_{26}NaN_2O_6$ $(MNa)^+$: 353.1683, found: 353.1705.

3.4.6. Alloc-Nle-OH. 73% yield as viscous oil; IR (KBr): 3329.9, 2959.1, 1715.5, 1537.8, 1456.4 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.47 (1H, bs, CO_2H), 6.34 (1H, d, J=8.5 Hz, NH), 5.95 (1H, ddt, J=16.5, 10.5, 5.5 Hz), CH_2=$CH$$CH_2O$), 5.34 (1H, dd, J=16.5, 1.5 Hz, CH_2=$CHCH_2O$), 5.25 (1H, dd, J=10.5, 1.5 Hz, CH_2=$CHCH_2O$), 4.61 (2H, d, J=5.5 Hz, CH_2=$CHCH_2O$), 4.39 (1H, m, CH), 1.89 (1H, m, $CH_2CH_2CH_2CH_3$), 1.73 (1H, m, $CH_2CH_2CH_2CH_3$), 1.37 (4H, m, $CH_2CH_2CH_2CH_3$), 0.93 (3H, t, J=7.0 Hz, $CH_2CH_2CH_2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 177.5, 156.0, 132.5, 118.0, 53.7, 66.0, 32.0, 27.2, 22.2, 13.8; HRMS (ESI) calcd for $C_{10}H_{17}NaNO_4$ $(MNa)^+$: 238.1049, found: 238.1035.

3.4.7. Alloc-Phe-OH. 84% yield as viscous oil; IR (KBr): 3329.9, 3042.7, 2947.0, 1718.6, 1522.2, 1492.6 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.32 (3H, m, ArH), 7.21 (2H, m, ArH), 6.27 (1H, d, J=8.0 Hz, NH), 5.91 (1H, ddt, J=17.0, 10.5, 5.5 Hz, CH_2=$CH$$CH_2O$), 5.26 (2H, m, CH_2=$CHCH_2O$), 4.72 (1H, dt, J=8.0, 6.0 Hz, CH), 4.59 (2H, d, J=5.5 Hz, CH_2=$CHCH_2O$), 3.24 (1H, dd, J=14.0, 6.0 Hz, CH_2), 3.14 (1H, dd, J=14.0, 6.0 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 176.3, 155.8, 135.5, 132.4, 129.3, 128.7, 127.3, 118.0, 66.0, 54.5, 37.7; HRMS (ESI) calcd for $C_{13}H_{15}NaNO_4$ $(MNa)^+$: 272.0893, found: 272.0910.

3.4.8. Alloc-Pro-OH. 84% yield as viscous oil; IR (KBr): 3102.6, 1704.3, 1450.4; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.59 (1H, bs, CO_2H), 5.90 (1H, m, CH_2=$CH$$CH_2O$), 5.34 (2H, m, CH_2=$CHCH_2O$), 4.65 (2H, m, CH_2=$CHCH_2O$), 4.41 (1H, m, N$CH$$CO_2H$), 3.55 (2H, m, N$CH_2CH_2CH_2$), 2.20 (2H, m, $NCH_2CH_2CH_2$), 1.98 (2H, m, $NCH_2CH_2CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: (155.7, 154.5), (177.9, 176.4), 132.5, (117.7, 117.3), (66.5, 66.2), (59.2, 58.6), (46.9, 46.6), (30.9, 29.4), (24.3, 23.4); HRMS (ESI) calcd for $C_9H_{14}NO_4$ $(MH)^+$: 200.0917, found: 200.0898.

3.4.9. Alloc-Ser(*t*Bu)-OH. 70% yield as viscous oil; IR (KBr): 3331.5, 2976.2, 1729.8, 1517.6; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.93 (1H, ddt, J=17.0, 10.5, 5.5 Hz, CH_2=$CH$$CH_2O$), 5.65 (1H, d, J=8.5 Hz, NH), 5.35 (1H, d, J=17.0 Hz, CH_2=$CHCH_2O$), 5.25 (1H, d, J=10.5 Hz, CH_2=$CHCH_2O$), 4.62 (2H, d, J=5.5 Hz, CH_2=$CHCH_2O$), 4.49 (1H, m, CH), 3.91 (1H, dd, J=9.0, 3.5 Hz, CH_2O*t*Bu), 3.61 (1H, dd, J=9.0, 3.5 Hz, CH_2O*t*Bu), 1.19 (9H, s, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.1, 156.1, 132.5, 118.0, 74.0, 66.0, 61.7, 54.2, 27.2; HRMS (ESI) calcd for $C_{11}H_{19}NaNO$ $(MNa)^+$: 268.1155, found: 268.1135.

3.4.10. Alloc-Val-OH. 85% yield as viscous oil; IR (KBr): 3329.9, 2970.9, 1707.6, 1534.3, 1468.3 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.34 (1H, d, J=8.5 Hz, NH), 5.95 (1H, ddt, J=17.0, 10.5, 5.5 Hz, CH_2=$CH$$CH_2O$), 5.34 (1H, dd, J=16.0, 1.0 Hz, CH_2=$CHCH_2O$), 5.25 (1H, dd, J=10.5, 1.0 Hz, CH_2=$CHCH_2O$), 4.61 (2H, d, J=5.5 Hz, CH_2=$CHCH_2O$), 4.36 (1H, dd, J=9.0, 4.4 Hz, CH), 2.26 (1H, m, $CH(CH_3)_2$), 1.03 (3H, d, J=7.0 Hz, $CH(CH_3)_2$), 0.96 (3H, d, J=7.0 Hz, $CH(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ:176.9, 156.3, 132.5, 118.0, 58.8, 66.1, 31.0, (18.99, 17.32); HRMS (ESI) calcd for $C_9H_{15}NaNO_4$ $(MNa)^+$: 202.1074, found: 202.1082.

3.5. Library synthesis

Rink amide resin (0.8 mmol/g) was loaded with a substoichiometric amount of *N*-α-Fmoc-*N*-ε-4-methyltrityl-lysine (0.2 mmol/g), DIC (1.0 equiv.), HOBt (1.0 equiv.) in DMF (10 mL/g) for 12 h and acetic anhydride (5.0 equiv.) with 2,6-lutidine (5.0 equiv.) was added to cap the resin (2 h). Four 280 mg portions of this resin were distributed into 5 mL tubes on an Argonaut Quest 210 and, after 30 min swelling in CH_2Cl_2, the resins were deprotected with 20% piperidine in DMF (3 mL) for 2.5 min. An automated washing procedure was used after each step involving four DMF washes (2 mL/g of resin) with a 30 s agitation (no contracting solvents such as methanol or diethyl ether were used). The resins were then treated with Fmoc-8-amino-3,6-dioxaoctanoic acid (2.0 equiv.), HOBt (4.0 equiv.) and DIC (4.0 equiv.) in DMF (2 mL) for 6 h. Subsequent Fmoc deprotection (20% piperidine in DMF, 3 mL) followed by coupling of each pool to an acrylic amino acids **10** (2.0 equiv.), DIC (4.0 equiv.), HOBt (4.0 equiv.) afforded four pools of intermediates **21**. The Fmocs were removed (20% piperidine in DMF, 3 mL) and each pool, in CH_2Cl_2 at 0 °C, was treated sequentially with Et*i*Pr_2N (4.0 equiv.) and Alloc-Cl (4.0 equiv.) for 30 min. The pools were then treated with a 1% TFA solution containing 5% Et_3SiH for 1 h to obtain the

polymer bound intermediates **23** which were encoded according to the general procedure below. The four pools were then combined for the Alloc deprotection according to the general procedure below and redistributed in ten 5 mL tubes. All resin manipulations and distributions were carried out as slurries using an isopycnic mixture of CH_2Cl_2 and DMF and an eppendorf repeater for distribution. The ten pools were subjected to their respective amino acid coupling followed by the encoding according to the general procedures to obtain ten pools of **28**. Two more iteration of this cycle led to 10 pool of intermediate **30** which were mixed, and Fmoc deprotected (20% piperidine) followed by treatment of fluoresein isothiocyanate (4.0 equiv.) and 2,6-lutidine in DMF (10 mL/g) for 5 h while shielding the reaction from light with aluminum foil to obtain after filtration and washing a distinctly yellow/orange polymer. This resin was treated with TFA–cresol (4:1, 10 mL) for 3 h then precipitated in Et_2O (200 mL) and pelleted by centrifugation (9800 g). The pellet was taken back up in neat TFA (7 mL) and reprecipitated and pelleted in Et_2O (200 mL). The pellet was washed several times with Et_2O than dissolved in a 1:1 mixture of AcCN–H_2O (1:1, 20 mL) and lyophilized to obtain 390 mg of a bright yellow powder.

3.5.1. General procedure for encoding steps. The resin was treated with 20% piperidine in DMF for 2.5 min, washed according to the previously described procedure and treated with a premixed (5 min) solution of PNA monomer **11** (4.0 equiv.), Et*i*Pr$_2$N (4.0 equiv.), HATU (3.5 equiv.) and 2,6-lutidine (6.0 equiv.) for 1 h. The coupling was repeated a second time then the resin was capped with acetic anhydride (5.0 equiv.) and 2,6-lutidine (5.0 equiv.) in DMF (10 mL/g). This cycle was repeated 4 times for the first and last codons (**23** to **27** and **29** to **30**) and 3 times for the second and third codons (**27** to **28** and **28** to **29**).

3.5.2. General procedure for Alloc deprotection. The resin was treated with a solution of $Pd(PPh_3)_4$ (0.2 equiv.), AcOH (10 equiv.) in CH_2Cl_2 (5 mL/g) followed by a solution of Et_3SiH in DMF (5 mL/g) for 30 min.

3.5.3. General procedure for DIC-mediated couplings. A solution of acid **25**, DIC (4.0 equiv.) and HOBt (4.0 equiv.) in DMF (10 mL/g of resin) was premixed for 5 min than added to the resin and the reaction was continued for 2 hr then capped by the addition of acetic anhydride (5.0 equiv.) and 2,6-lutidine (5.0 equiv.).

4. Supplementary Material

Mass spectroscopy data of compounds **27–29** as well as other library intermediates.

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Combinatorial solid-phase synthesis and screening of a diverse tripodal triazacyclophane (TAC)-based synthetic receptor library showing a remarkable selectivity towards a D-Ala-D-Ala containing ligand

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Abstract—A large and diverse library of a TAC-based tripodal synthetic receptor library (**6**) has been prepared by split-mix synthesis on the solid phase. Each receptor of the 46,656-member TAC-based library (**6**) is attached to a solid support bead and contains three different dipeptide arms. On-bead screening for binding of a D-Ala-D-Ala containing ligand (**7**) by the TAC-based library (**6**) was performed in phosphate buffer (0.2 N, pH=7.0). Remarkable selectivity for particular library members was observed. The best binding members from the screening were manually selected using fluorescence microscopy and subjected to structural analysis by sequencing. The thus determined binding sequences showed a high consensus.

1. Introduction

Design and synthesis of synthetic receptors molecules for selective recognition of bioactive molecules have received considerable attention in recent years.[1] Synthetic receptors for specific peptide sequences may provide model systems for biologically relevant peptide-protein interactions and may lead to applications in the area of biosensors, therapeutics and catalysis. Among the peptide containing receptors, the well-studied tweezer-like synthetic receptors that contain two binding arms showed high selectivity for certain peptide sequences.[2,3] Since amino acid sequences of the receptor arms are crucial for the recognition of a ligand, one-bead-one-compound combinatorial solid phase synthesis,[4] provides a powerful strategy for the generation of libraries of synthetic receptors with attractive affinity and/or selectivity for specific peptide sequences.[5] Preparation of a library on the solid phase using split-mix synthesis[6] ensures that each single bead carries one particular receptor. As a result, the library can be screened either manually or using a bead sorter and the selected receptor beads can be subjected to structural elucidation.

In order to increase diversity, possibly affinity and selectivity, we are interested in the development of tripodal synthetic receptors that contain—compared to tweezers—an additional peptide-binding arm. Only a limited number of tripodal scaffolds suitable for attachment of three binding arms have been described so far.[7] We are particularly interested in tripodal triazacyclophane (TAC) and cyclotriveratrylene (CTV) scaffolds and we have developed efficient syntheses for both.[5a,8] In addition, their applicability for the preparation of tripodal receptors with two or three identical peptidic arms has been described.[5a,8] Receptors were uncovered with promising selectivity and binding affinity towards binding of bacteria cell wall precursors containing D-Ala-D-Ala or D-Ala-D-Lac[5a,9] sequences. In order to significantly increase the structural diversity, a selectively deprotectable TAC scaffold (**1**)[10] was developed. The presence of orthogonal protecting groups such as fluorenylmethoxycarbonyl (Fmoc), 2-nitrobenzenesulfonyl (*o*-NBS), and allyloxycarbonyl (Aloc) allowed the selective introduction and elongation of three

Keywords: Synthetic receptors; TAC-based library; Screening; Fluorescent ligand; D-Ala-D-Ala-OH.

Abbreviations: Aloc, Allyloxycarbonyl; Boc, *tert*-butoxycarbonyl; BOP, benzotriazol-tris-(dimethylamino)phosphonium hexafluoro-phosphate; tBu, *tert*-butyl; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DCM, dichloromethane; DiPEA, *N*,*N*-diisopropyl-*N*-ethylamine; DMF, dimethylformamide; Et_2O, diethyl ether; Fmoc, *N*-fluoren-9-ylmethoxycarbonyl; HOBt, 1-hydroxybenzotriazol; *o*-NBS, 2-nitrobenzenesulfonyl; NMP, *N*-methylpyrrolidone; rt, room temperature; TAC, 1,5,9-triaza-3(1,3)-benzenacyclododecaphane-3^5-carboxylic acid; TEA, triethylamine; TFA, trifluoroacetic acid; TIS, triisopropylsilane.

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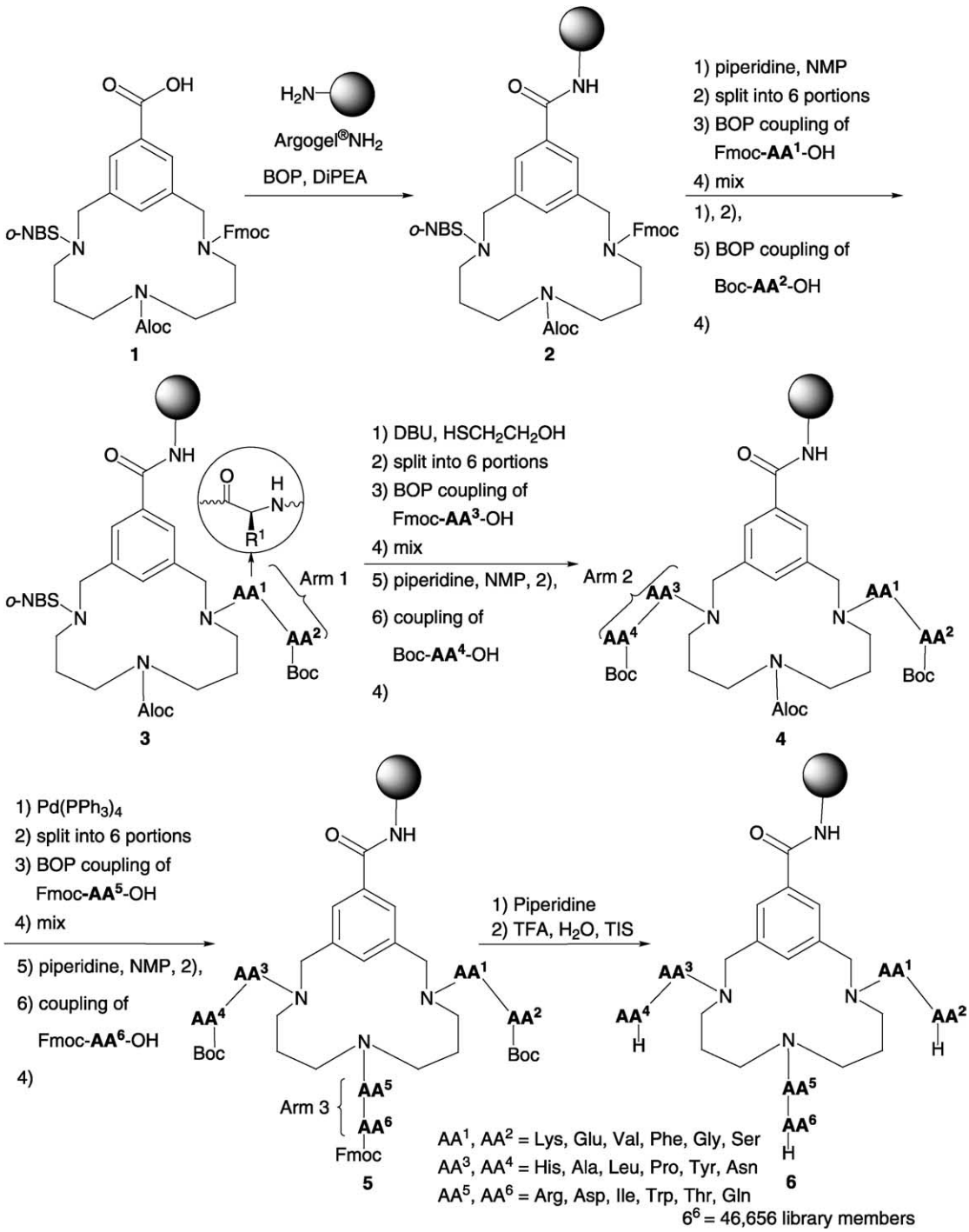
different peptide arms.[11] The suitability of **1** for the construction of TAC-based receptors was recently demonstrated by the preparation on the solid phase of diverse TAC-based libraries consisting of a considerable number of library members having three different amino acids and/or dipeptides as binding arms (theoretically 225 and 27,000 members, respectively).[11]

Here, we describe the construction of a diverse and large combinatorial library of TAC-based tripodal synthetic receptors on the solid phase. As an example for selective recognition of peptide sequences, the 46,656-member TAC-based receptor library was screened for binding of the D-Ala-D-Ala sequence.

2. Results and discussion

2.1. Solid phase synthesis of the TAC-based library 6

A TAC-based tripodal receptor library (**6**) containing three different peptide arms was prepared using the split-mix method.[4,6] In order to facilitate screening experiments, the TAC-based receptors were covalently bound to



Scheme 1. Combinatorial solid phase synthesis of a large and diverse triazacyclophane (TAC)-based synthetic receptor library consisting of 46,656 members.

Argogel®-NH_2 resin. The strategy used for the preparation of library **6** is depicted in Scheme 1. The Argogel®-NH_2 resin was loaded with TAC scaffold (**1**) and the three different dipeptide arms (Arm 1, Arm 2, and Arm 3) were assembled one by one onto the TAC scaffold in two cycles after subsequent cleavage of the corresponding orthogonal protecting group. To ensure the possibility of complete structural elucidation by Edman degradation[12] by having unique amino acid sequences for each library member, three different representative sets each containing six different amino acids were chosen for the construction of the three peptide arms (Scheme 1). The side chains of functional amino acids were protected with acid-labile groups. Eighteen out of the twenty proteinogenic amino acids were used excluding cysteine and methionine. Fmoc-protected amino acids were used as a building block for the first cycle whereas Boc-protected amino acids were used for the second cycle in order to prevent further elongation. In the last, third arm, Fmoc-protected amino acids were used for the second coupling cycle to determine the final average loading of the library.

Table 1. Sequences of the red beads

Bead	Arm 1		Arm 3		Arm 2	
	AA^2	AA^1	AA^6	AA^5	AA^4	AA^3
1[a]	Ser	Gly	Ile	Thr	His	His
2[a]	Lys	Ser	Thr	Gln	His	His
3[a]	Phe	Gly	Thr	Gln	His	His
4[a]	Phe	Gly	Thr	Thr	His	His
5[a]	Val	Lys	Thr	Ile	His	His
6[b]	Val	Glu	Arg	Arg	His	His
7[b]	Phe	Lys	Trp	Arg	His	His

[a] Dark-red bead.
[b] Light-red bead.

Thus, loading of Argogel®-NH_2 resin using BOP[13] as a coupling agent led to resin **2**. After removal of the Fmoc group, the resin was divided into six equal portions and BOP-coupling of the corresponding Fmoc-protected amino acid of Set 1 [Fmoc-L-Lys(Boc)-OH, Fmoc-L-Glu(O^{t}Bu)-OH, Fmoc-L-Val-OH, Fmoc-L-Phe-OH, Fmoc-Gly-OH, Fmoc-L-Ser(^{t}Bu)-OH)] was performed. Negative chloranil[14] and bromophenol blue (BPB)[15] tests for the presence of secondary amines confirmed completion of the coupling reactions in all the cases. After mixing of the six portions, the Fmoc group was removed and the resin was split again into 6 portions. After BOP-coupling of the second amino acid, using the corresponding Boc-protected amino acids of Set 1 [Boc-L-Lys(Boc)-OH, Boc-L-Glu(O^{t}Bu)-OH, Boc-L-Val-OH, Boc-L-Phe-OH, Boc-Gly-OH, Boc-L-Ser(^{t}Bu)-OH)], pooling was carried out to give library **3**. The *o*-NBS group was removed from the pooled resin by thiolysis[16] and introduction and synthesis of the second arm (Arm 2) was performed analogously to the construction of the first arm. In this case, amino acid from Set 2 [Fmoc-L-His(Trt)-OH, Fmoc-L-Ala-OH, Fmoc-L-Leu-OH, Fmoc-L-Pro-OH, Fmoc-L-Tyr(^{t}Bu)-OH, Fmoc-L-Asn(Trt)-OH] and their Boc-protected counterparts were used to afford **4**. After pooling, the Aloc group was removed by Pd-catalyzed allyl transfer to anilinium *p*-toluenesulfinate[10,17] and the third arm (Arm 3) was introduced similarly, using only Fmoc-protected amino acids from Set 3 [Fmoc-L-Arg(Pbf)-OH, Fmoc-L-Asp(O^{t}Bu)-OH, Fmoc-L-Ile-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-L-Thr(^{t}Bu)-OH, Fmoc-L-Gln(Trt)-OH], to give the fully protected TAC-based library **5**.

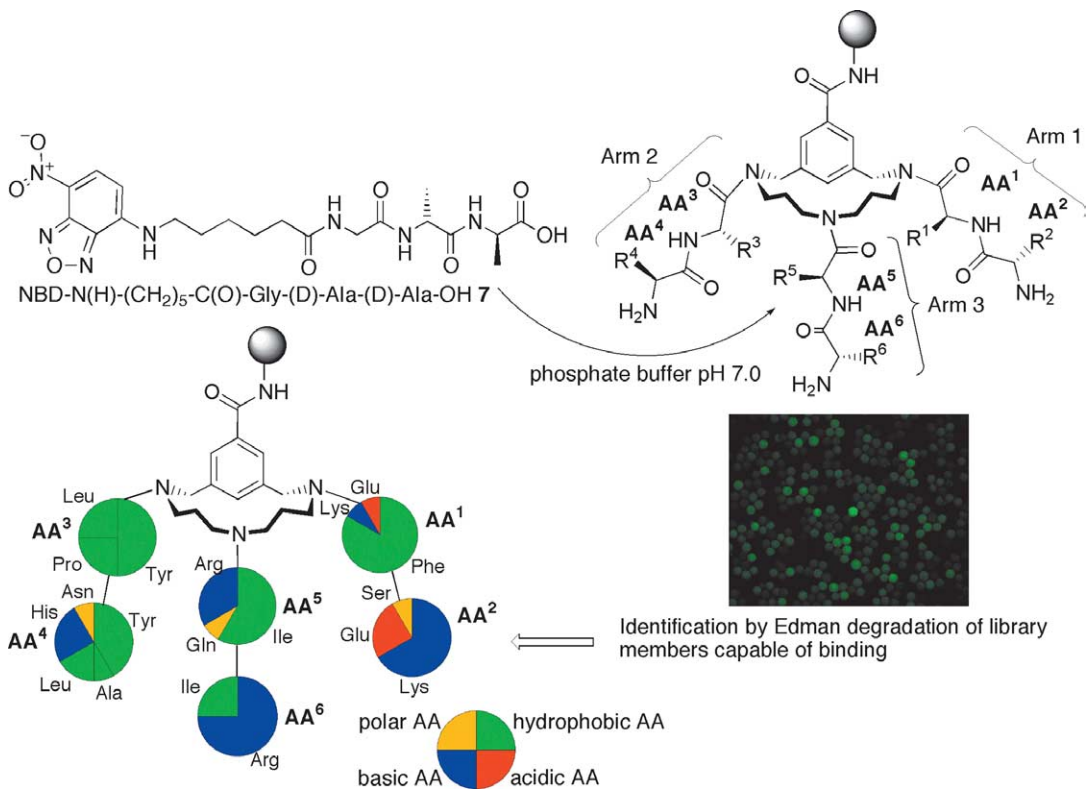


Figure 1. Screening results of the TAC-based library with a fluorescent D-Ala-D-Ala containing ligand and visualization of the found amino acids at each position in the peptide binding arms by a 'pie' representation.

Finally, Fmoc-cleavage followed by global deprotection of all functionalized side chains as well as Boc cleavage using TFA/TIS/H_2O was performed and the fully deprotected TAC-based library **6** was obtained. The large and highly diverse library, containing 46,656 different receptors (6^6), was now ready for screening of selective binding by specific peptide sequences. Interestingly, a low percentage of red-colored beads were found. To determine the identity of those beads, five dark red beads together with two light-red beads were selected and sequenced showing the presence of His-His sequence (AA^3-AA^4) in one of the outer arms (Arm 2) (Table 1, beads 1–7). Apparently the red color was associated with the presence of His-His sequence in outer arm 2 (AA^3, AA^4). Since a Pd^0-catalyst was used for Aloc cleavage, a complex of Pd^{2+} and the His-His sequence,[18] might have caused the red color. Only the dark-red beads showed also the presence of Thr in the middle arm 3 (AA^5-AA^6), indicating that the intensity of the red color might depend on the amino acid composition of the middle arm (Table 1).

2.2. Screening for binding of D-Ala-D-Ala by the TAC-based library 6

As part of a program towards uncovering compounds capable of binding to crucial structural elements of pathogens, peptide sequence D-Ala-D-Ala present in the peptidoglycan precursor needed for construction of the cell wall in growing gram-positive bacteria[9] was chosen as a ligand for assessing the binding properties of TAC-based receptor library **6**. This might ultimately lead to synthetic receptors capable of mimicking the binding and thereby antibiotic properties of vancomycin.[19] In order to visualize the best binding beads by screening, a ligand containing the fluorescent NBD label, that is, NBD-N(H)-$(CH_2)_5$-C(O)-Gly-D-Ala-D-Ala-OH (**7**)[20] was used (Fig. 1).

Thus, screening for the ability to bind the D-Ala-D-Ala containing ligand (**7**) by the TAC-based tripodal receptor library **6** was performed in phosphate buffer (0.2 N, pH=7.0) (Fig. 1) on ca 140,000 beads, roughly corresponding to three copies of each synthetic receptor. So far there are not many examples of screening for binding by synthetic receptors in aqueous systems.[21] However, this was deemed absolutely essential if one wants to move to biologically relevant systems and find hits for these. We found that it is no longer difficult to find hits of synthetic receptors with good to excellent binding properties in organic solvents[22] The challenge, however, is to discover molecules with good binding properties in an aqueous environment, since intermolecular interactions (hydrogen bond, electrostatic) between ligand and receptor are much weaker here than in apolar solvents.

Upon incubation with fluorescent ligand **7** a high selectivity of binding was found as was judged by observation of a range of intensities of fluorescent beads under the fluorescence microscope (Fig. 1). The most intensive twelve fluorescent beads were selected and subjected to Edman degradation.[12] As a confirmation of the identity of the synthetic receptors, Edman sequencing was carried out in all cases for an additional third cycle, which invariably showed the absence of a third amino acid, thus confirming the length of the peptide arms. As a negative control, a non fluorescent bead was selected and sequenced. The identity of the peptide arms of the bead attached TAC-based receptors is shown in Table 2. Perusal of the data revealed a remarkable selectivity. First, completely different sequences were obtained for the negative control as compared to the fluorescent beads. Interestingly, identical or very similar amino acid sequences were found in the receptors present on the selected fluorescent beads. Despite the fact that histidine was found at position AA^3 or AA^4 in some of the fluorescent beads, none of them were colored red (vide supra) indicating that the His-His sequence was required for this property (Table 1). The frequency of the found amino acids at each position is shown with a 'pie' representation of each amino acid residue (Fig. 1). From Table 2 the following trends can be inferred. Basic amino acids (blue) were especially found at the end of the arms (AA^2: Lys 67%, AA^4: His 25%, AA^6: Arg 75%) and predominantly in arms 1 (Lys) and 3 (Arg). Arg was also found at AA^5(33%) in arm 3. Hydrophobic amino acids (green) were often found closest to the scaffold (AA^1: Phe 83%, AA^3: Leu/Pro 50% and Tyr 50%, AA^5: Ile 58%). However, AA^4 had usually a fairly hydrophobic character, and Tyr, Ala and Leu accounted for 67% of the amino acids in the sequenced beads at his position. Combination of a basic amino acid and a hydrophobic amino acid was especially prominent in arms 1 and 3.

Table 2. Sequences of synthetic receptors for **7**

Bead	Arm 1		Arm 3		Arm 2	
	AA^2	AA^1	AA^6	AA^5	AA^4	AA^3
8[a]	Lys	Phe	Arg	Ile	His	Tyr
9[a]	Lys	Phe	Arg	Ile	Tyr	Tyr
10[a]	Lys	Phe	Arg	Ile	His	Pro
11[a]	Lys	Phe	Arg	Ile	Ala	Leu
12[a]	Glu	Phe	Arg	Ile	Tyr	Pro
13[a]	Glu	Lys	Arg	Ile	Tyr	Leu
14[a]	Lys	Phe	Arg	Arg	Tyr	Leu
15[a]	Lys	Phe	Arg	Arg	Leu	Tyr
16[a]	Lys	Phe	Ile	Arg	Leu	Tyr
17[a]	Ser	Glu	Ile	Arg	His	Pro
18[a]	Lys	Phe	Ile	Ile	Tyr	Tyr
19[a]	Glu	Phe	Arg	Gln	Asn	Tyr
20[b]	Phe	Phe	Asp	Gln	Asn	His

[a] Fluorescent bead.
[b] Non-fluorescent, control bead.

Remarkable is the number of times an identical sequence was found in arm 1 or arm 3. Lys-Phe was found in arm 1 in 8 out of 12 sequenced beads, that is, 66%. Arg-Ile in arm 3 was found in 6 out of 12 beads, that is, 50%. The combination of the these two sequences, that is, Lys-Phe in arm 1 and Arg-Ile in arm 3 was even found in 4 out of 12 beads, that is, in 33% of the sequenced beads.

These data point to remarkable selectivity of binding, since this combination sequence is only present in 36 (6^2: possible combinations of amino acids in arm 2) library members in the total library of 46,656, corresponding to only 0.08% of the beads.

Based on these data it is tempting to speculate about a possible binding mode of ligand **7** to a 'cavity' formed by arm 1 and arm 3 in the sequenced synthetic receptors.

Hydrophobic interactions or aromatic π-stacking might be possible between the fluorescent label of the ligand (NBD group) and the branched or aromatic side chains of predominantly Phe (AA^1) in arm 1 or Tyr (AA^3) in arm 2 and Ile (AA^5) in arm 3, which are located close to the TAC-scaffold. Ionic interactions are likely between the Ala-carboxylate terminus of ligand **7** and the amino termini and/or the basic side chains of outer amino acids Lys (AA^2) in Arm 1 and Arg (AA^6) in Arm 3. Although Arm 2 seemed in this model to be less involved in the binding, which was also perceptible from the higher variability of amino acids in this arm, hydrogen bond formation with the backbones amide bonds and/or hydrophobic interactions with the Ala-Me groups are possible. As a consequence the role of hydrophobic amino acids might also be to create a hydrophobic environment in the aqueous medium of screening, which is favorable for binding. Such a situation is distantly related to the hydrophic cavities created by many enzymes.[23]

3. Conclusions

A convenient split-mix protocol was used for the preparation of a large and diverse synthetic receptor library based on the TAC-scaffold, which allowed the introduction of three different (peptide) arms. Although of considerable size (46,656 members), the size of the library can be easily increased by the introduction of longer arms and arms containing up to ten amino acids have been introduced. Screening for binding of the fluorescent D-Ala-D-Ala containing ligand (**7**) by the tripodal receptor library **6** was carried out in phosphate buffer aqueous system and led to the identification of selective synthetic receptors.

Under present investigation is the preparation of larger libraries and screening with other ligands.

4. Experimental

4.1. General

All reagents were purchased from commercial sources and used without further purification. Argogel®-NH_2 (0.40 mmol/g, average bead diameter 178 μm) resin was purchased from Argonaut Technologies, Inc. Protected amino acids were purchased from Alexis Corporations (Läufelfingen, Switzerland) and Advanced Chemtech Europe (UK). All reactions on the solid phase were performed in standard glassware or poly(ethylene) glycol (PE) syringes with PE frits. Peptide grade solvents, dried on molecular sieves were used for reactions and resin washing steps. The used capping reagent was a mixture of acetic anhydride (42 mL), DiPEA (2.18 mL), HOBT (0.23 g), and NMP (100 mL). Anilinium *p*-toluenesulfinate[24] was obtained by reaction of *p*-toluenesulfinic acid sodium salt with aniline in DCM and crystallized upon slow adition of hexane. DiPEA was subsequently distilled from KOH and ninhydrin. For Fmoc determinations, a Perkin Elmer Lambda 2 UV/VIS spectrometer was used. Kaiser,[25] bromophenol blue (BPB),[15] and chloranil tests[14] were used for detection of remaining primary and/or secondary amines on the solid phase. Edman degradations[12] were performed on an Applied Biosystems ABI 476A protein sequencer. Polymer beads were visualized and manipulated under a Leica MZ FL III microscope equiped with a CCD camera.

4.2. General procedure for coupling Fmoc/Boc-amino acids on the solid phase

The resin (1 equiv.) was swollen in NMP (2 min) and, after draining the solvent, BOP (4 equiv.), Fmoc/Boc-amino acid (4 equiv.) and NMP (15 mL/mmol) were added. The mixture was shaken until complete disolution and DiPEA (8 equiv.) was added. After shaking for 4 h, the resin was washed with NMP (5×7 mL, each 2 min) and DCM (5×8 mL, each 2 min). Negative Kaiser, BPB tests and/or chloranil test indicated completion of the coupling reaction. After drying the resin in vacuo overnight, loading of the resin was assessed by Fmoc-determination.

4.3. General procedure for N^{α}-Fmoc deprotection

N^{α}-Fmoc-protected resin was swollen in NMP (2 min) and, after draining the solvent, the resin was shaken with 20% piperidine in NMP (3×10 mL/mmol, each 10 min). The resin was washed with NMP (5×2 min) and DCM (5×2 min). Positive Kaiser, BPB and/or chloranil tests indicated the successful Fmoc-deprotection.

4.4. Solid phase synthesis of library 6

Argogel®-NH_2 (6.25 g, 2.5 mmol) was swollen in NMP (38 mL, 2 min). After draining the solvent, **1** (1.92 g, 2.5 mmol), BOP (1.10 g, 2.5 mmol) and NMP (38 mL) were added and a gentle stream of dry nitrogen was bubbled though the mixture until all reagents were dissolved. DiPEA (0.87 mL, 5 mmol) was added and N_2 bubbling was continued overnight. The resin was drained and washed with NMP (5×38 mL, each 2 min), DCM (5×38 mL, each 2 min) and Et_2O (5×38 mL, each 2 min). After drying in vacuo overnight, the loading of the resin was determined (0.33 mmol g^{-1}). Remaining free amines on the resin were acetylated by addition of the capping agent (15 mL/mmol) and shaking for 1 h. After draining, the resin was washed with NMP (5×38 mL, each 2 min) and DCM (5×38 mL, each 2 min). After N^{α}-Fmoc deprotection, resin **2** was dried under vacuo overnight and divided into six equal portions (1.13 g, 0.33 mmol) in PE syringes with PE frits. each resin portion was swollen in NMP (5 mL, 2 min) and after draining the solvent, coupling of a different Fmoc-amino acid from Set 1 for AA^1 [Fmoc-L-Lys(Boc)-OH, Fmoc-L-Glu(O^{t}Bu)-OH, Fmoc-L-Val-OH, Fmoc-L-Phe-OH, Fmoc-Gly-OH, Fmoc-L-Ser(^{t}Bu)-OH)] in each syringe was carried out following the general procedure. The average loading was calculated from each individual loading and was 0.32 mmol.g^{-1}. The content of the syringes was pooled and mixed in a reaction vessel, washed with NMP (2×30 mL, each 2 min) and N^{α}-Fmoc was cleaved following the general protocol. Then, the split-mix procedure was repeated for the coupling of the second amino acid. The corresponding Boc-amino acid from Set 1 for AA^2 [Boc-L-Lys-(Boc)-OH, Boc-L-Glu(O^{t}Bu)-OH, Boc-L-Val-OH, Boc-L-Phe-OH, Boc-Gly-OH, Boc-L-Ser(^{t}Bu)-OH)] were

used to afford library **3**. Upon drying in vacuo overnight, **3** (2.08 mmol) was washed with DMF (5×30 mL, each 2 min) and dry (molecular sieves) DMF (5×30 mL, each 2 min). The solvent was drained again and the *o*-NBS group was cleaved by addition of DMF (30 mL), DBU (1.57 mL, 10.40 mmol, 5 equiv.) and β-mercaptoethanol (1.46 mL, 20.80 mmol, 10 equiv). After N_2 bubbling for 30 min, the green solution was replaced by a fresh mixture of identical composition and N_2 bubbling was maintained for another 30 min. The resin was washed with NMP (5×30 mL, each 2 min), DCM (5×30 mL, each 2 min) and Et_2O (5×30 mL, each 2 min) and dried under vacuo overnight. The split-mix procedure described above for the construction of the first arm was repeated for the second arm, using amino acids from Set 2 [Fmoc-L-His(Trt)-OH, Fmoc-L-Ala-OH, Fmoc-L-Leu-OH, Fmoc-L-Pro-OH, Fmoc-L-Tyr(^{t}Bu)-OH, Fmoc-L-Asn(Trt)-OH] for AA^3 or for AA^4 their Boc-protected analogues to give library **4**. lastly, the Aloc group was cleaved. After swelling of library **4** (2.0 mmol) in NMP (30 mL, 2 min) and draining the solvent, anilinium *p*-toluensulfinate (9.92 g, 40 mmol, 20 equiv) and NMP (30 mL) were added. A gentle stream of dry nitrogen was bubbled though the mixture for 5 min and tetrakis(triphenylphosphine)-palladium(0) (0.35 g, 0.3 mmol, 15 mol%) was added. N_2 bubbling was maintained for 1 h under exclusion of light. The resin was washed with NMP (3×30 mL, each 2 min), 0.1% solution of sodium diethyldithiocarbamate trihydrate in NMP (1×30 mL, each 2 min), a 20% solution of DiPEA in NMP (1×30 mL, each 2 min), NMP (5×30 mL, each 2 min), DCM (4×30 mL, each 2 min) and Et_2O (5×30 mL, each 2 min) and dried under vacuo overnight. For the construction of the third arm, only Fmoc-protected amino acid from Set 3 for both AA^5 and AA^6 [Fmoc-L-Arg(Pbf)-OH, Fmoc-L-Asp(O^{t}Bu)-OH, Fmoc-L-Ile-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-L-Thr(^{t}Bu)-OH, Fmoc-L-Gln(Trt)-OH] were used to furnish library **5** having an average loading of receptors equivalent to 0.32 mmol g^{-1} as was assessed by Fmoc-determination. After final pooling, the resin was washed with NMP (5×30 mL, each 2 min), DCM (5×30 mL, each 2 min) and Et_2O (5×30 mL, each 2 min) and stored in vacuo.

A small portion of the library **5** (0.25 g, 0.08 mmol) was subjected to global deprotection by Fmoc-deprotection, followed by treatment of the resin with a mixture of TFA: H_2O: TIS, 95:2.5:2.5 (v/v/v) (5 mL) for 4 h. The resulting resin was washed with NMP (3×1.2 mL, each 2 min), acetic acid (0.1 M) (3×1.2 mL, each 30 min), NMP (5×1.2 mL, each 2 min), 25% DiPEA in NMP (5×1.2 mL, each 2 min), NMP (5×1.2 mL, each 2 min), DCM (5×2 mL, each 2 min), dioxane (4×1.2 mL, each 2 min), dioxane: H_2O, (1:1) (4×1.2 mL, each 2 min), H_2O (4×1.2 mL, each 2 min), and Et_2O (4×1.2 mL, each 2 min), to give the fully deprotected library **6**.

4.5. Screening for binding of NBD-N(H)-$(CH_2)_5$-C(O)-Gly-D-Ala-D-Ala-OH by the TAC-based library

TAC-based synthetic receptor library **6** (0.18 g, 0.079 mmol, ~140.000 beads, ~3 copies/receptor) was suspended in a 46 μM solution of NBD-N(H)-$(CH_2)_5$-C(O)-Gly-D-Ala-D-Ala-OH (**7**) in phosphate buffer (0.2 N, pH=7.0) (158 mL) and incubated with gentle shaking and exclusion of light at 20 °C for 65 h. Then resin was drained and washed with phosphate buffer (0.2 N, pH=7.0) (5×158 mL, each 2 min). Then, resin was poured into a petri dish and spread into a monolayer. The beads were viewed under a fluorescence microscope. By use of a long needle, most fluorescent or colored beads were isolated (~180 beads). The fluorescence of these preselected beads was reevaluated using the overexposure mode of the Leica DC-100 digital camera system and image analysis to estimate the relative fluorescence intensities semi-quantitatively. The best twelve fluorescent beads were selected and analysed by parallel Edman degradation together with a non-fluorescent bead and four of the found red beads.

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24. After dissolving *p*-toluensulfinic acid sodium salt trihydrate (15 g) in boiling water (250 mL), HCl (1 N, 85 mL), was added and the reaction was cooled to rt. The *p*-toluensulfinic acid crystals were filtrated, washed with ice–water and dried. *p*-Toluensulfinic acid (4 g) was dissolved in DCM (25 mL) and aniline (2.33 mL) was added. Anilinium *p*-toluensulfinate crystals were obtained by slow addition of hexanes.
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Click chemistry on solid support: synthesis of a new REM resin and application for the preparation of tertiary amines

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Abstract—1,3-Dipolar cycloaddition was employed for the synthesis of a highly practical REM resin. Exploiting this concept, the resulting triazolylmethyl acrylate (TMA) resin was used for an efficient parallel synthesis of tertiary amines by Michael addition, subsequent quarternization and cleavage by means of β-elimination.

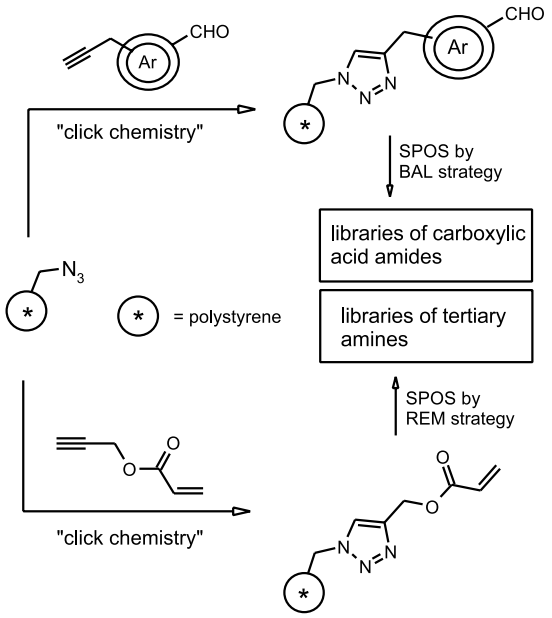
1. Introduction

Solid phase organic synthesis (SPOS) has become an area of huge interest in the field of organic and medicinal chemistry.[1] In contrast to solid phase peptide synthesis (SPPS), the application of SPOS comprises a broad variety of different organic reactions. There is an increasing demand to establish a great number of linking strategies in order to translate reaction types from solution to solid phase.[2] Ideally, the linker should be traceless, recyclable and easily attachable to a polystyrene based backbone. Typically, SPOS handles are connected to aminomethyl substituted polystyrene via a carboxylic function resulting in a secondary amide bearing a free NH that can be crucial for some reaction conditions during the SPO synthesis. On the other hand, linker immobilization by ether formation albeit leading to a widely inert functionality is hard to monitor. Thus, there is still need for the development of new linking strategies.

Very recently, we reported on the construction and application of a new family of backbone amide linkers (BAL) that were accessible by 1,3-dipolar cycloaddition of azidomethyl polystyrene and a propargyl or propargyloxy substituted arene carbaldehyde (Scheme 1) when the triazole formation was high yielding and conveniently monitored by IR spectroscopy.[3]

The resulting click linkers were applied for the parallel synthesis of dopaminergic carboxamides. As we were convinced that this click chemistry strategy[4,5] is suitable for the immobilization of a variety a different SPOS handles, we intended to exploit the methodology for the construction of further linkers.

Recently, J. R. Morphy and co-workers introduced the new acrylate- and vinylsulfone-based linkers **1** and **2**, which are in fact regenerative Michael acceptors (REM) being useful for the efficient synthesis of tertiary amines.[6–8] At this, addition of a secondary amine followed by quarternization of the resulting Michael product and subsequent cleavage led to the desired tertiary amines. Following this REM



Scheme 1. Synthesis and application of click chemistry derived BAL and REM resins.

Keywords: Click chemistry; REM Resin; 1,3-Dipolar cycloaddition; SPOS.
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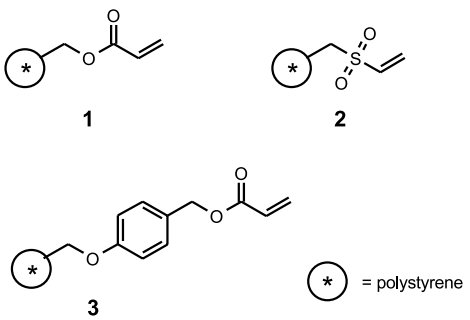
strategy, the acrylate **1** and the Wang resin based analog **3** were applied for the synthesis of δ-opioid receptor ligands,[9] β-peptoides,[10] potential I_f channel blockers[11] and Gly T2 transporter ligands (Scheme 2).[12]

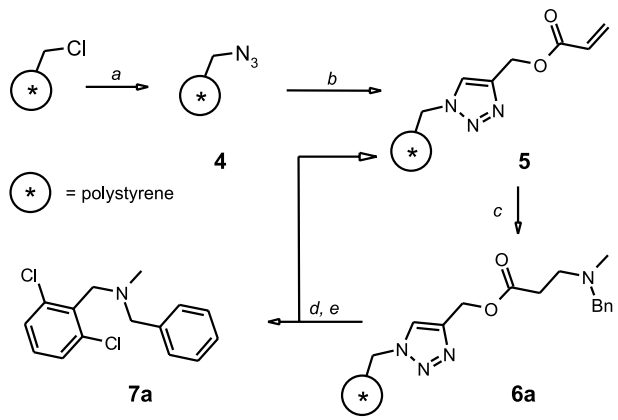


Scheme 2. Commercially available REM resins.

2. Results and discussion

In conjunction with our efforts in the discovery of highly selective dopamine receptor modulators, we are especially interested in tertiary amines being known as a valuable pharmacophoric functions. Thus, we decided to choose a REM based methodology for the extension of our studies on the application of click chemistry for the immobilization of linking units. In detail, we started from azidomethyl substituted polystyrene **4**, which was easily prepared by nucleophilic substitution of Merrifield resin with NaN_3.[13] The subsequent 1,3-dipolar cycloaddition with commercially available propargyl acrylate[14] was conducted in DMF/THF in the presence of DIPEA and catalytic amounts of Cu(I)I leading to the triazolylmethyl acrylate (TMA) resin **5** (Scheme 3). Addition of benzylmethylamine proceeded smoothly in DMF at room temperature. Subsequent alkylation of the aminopropionate **6a** with 2,6-dichlorobenzyl bromide was followed by base induced cleavage leading to the tertiary amine **7a** in 68% yield based on the loading of **5**. ^{1}H NMR analysis of the final product indicated a purity >95%. It is worthy of note that the use of a regenerated resin **5** for another identical reaction cycle resulted in the formation of **7a** in almost equal yield (66%).

Figure 1 documents a diagnostic IR analysis when both the azido and the ester C═O functionality reveal strong



Scheme 3. (a) NaN_3, DMSO, 70 °C, 48 h; (b) propargyl acrylate, Cu(I)I, DMF, THF, DIPEA, 35 °C, 10 h; (c) benzylmethylamine, DMF, rt, 16 h; (d) 2,6-dichlorobenzyl bromide, DMSO, rt, 16 h; (e) TEA, DMF, rt, 16 h.

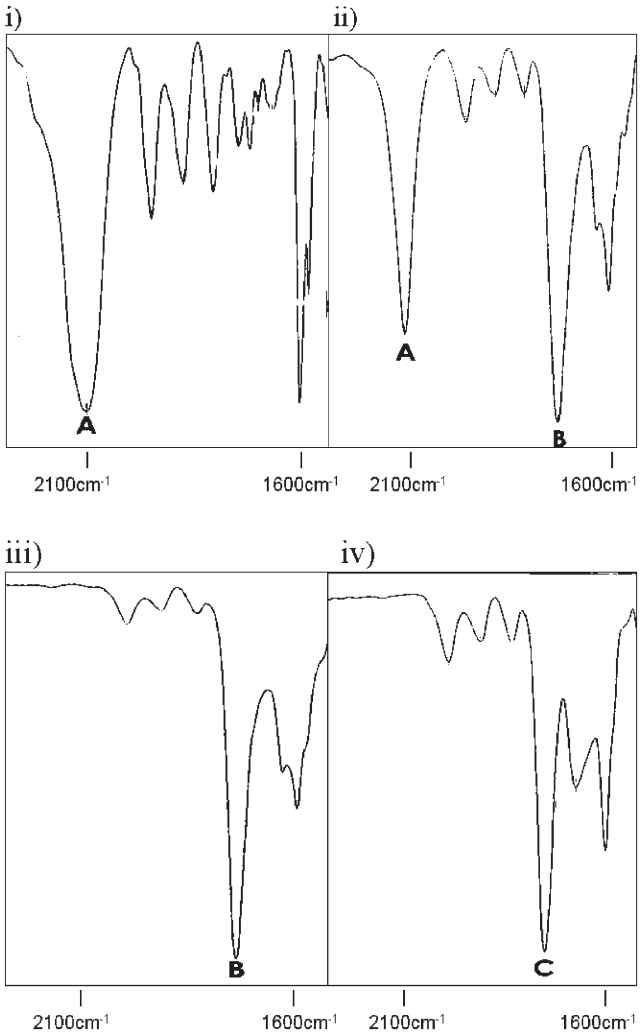


Figure 1. IR monitoring of solid phase reactions. All spectra range from 2300 to 1500 cm^{-1}. (i) Resin **4** with azido band at 2096 cm^{-1} (**A**); (ii) sample after 5 h reaction time of **4** with propargyl acrylate still showing an azido band at 2096 cm^{-1} (**A**) and a signal for the acrylic ester moiety at 1732 cm^{-1} (**B**); (iii) sample after 10 h reaction time of **4** with propargyl acrylate displaying the acrylic ester signal at 1732 cm^{-1} (**B**) and complete disappearance of the azido group; (iv) resin **6a** with a slightly shifted ester signal at 1735 cm^{-1} (**C**).

IR absorption. The progression of the cycloaddition can be monitored conveniently and precisely. After 5 h of reaction time, the signal of the azido group (2096 cm^{-1}) has been distinctly shrunk whereas a carbonyl band (1732 cm^{-1}) becomes visible. Another 5 h of reaction time leads to a complete disappearance of the N_3-signal. The 1,4-addition of an amine results to a slight shift the carbonyl band, whereas the E1cb elimination regenerated the acrylate resin showing IR absorption at 1732 cm^{-1} (not shown).

In order to evaluate our new TMA resin and to compare it to the off-the-shelf REM support **1**, we accomplished a parallel synthesis of six tertiary amines. In detail, the Michael acceptor **5** was treated with tetrahydroisochinoline or phenylpiperazine to give the Michael products **6b** and **6c**, respectively. Subsequent alkylation with allyl bromide, *p*-nitrobenzyl bromide or methyl bromoacetate followed by Hofmann elimination gave the tertiary amines **7b–f**, respectively.

Employing phenylpiperazine as a secondary amine and methyl bromoacetate an electrophile, the reaction led to the 4-bromophenylpiperazine **8** when LCMS indicated a product of high purity and the mass signals of m/z=313 ($M+1^+$) and 315 ($M+3^+$) revealed the typical isotope pattern of bromine. ^{1}H NMR spectra indicated an AA'BB' coupling pattern for the protons of the phenyl moiety. Being known that 2-haloacetonitriles can act as efficient halogenating reagents,[15] we assume that the excess of bromoacetate reacted analogously, in this case.

For all SPOS products investigated, the yields were satisfying and comparable to those described in the literature based on resin **1** (Table 1). LCMS analysis employing reversed phase chromatography in combination with UV (220 nm) and ESI-ion trap detection indicated excellent purities (>92%).

In conclusion, a convenient and practical construction of a triazolylmethyl acrylate (TMA) resin employing 1,3-dipolar cycloaddition as the key immobilization step facilitates an efficient parallel synthesis of differently substituted tertiary amines. Application for a combinatorial approach to selective dopamine receptor modulators is currently in progress.

3. Experimental

3.1. General

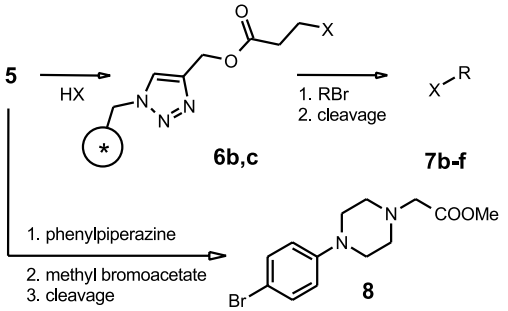
Solvents and reagents were purified and dried by standard procedures or purchased in pure, dry quality from Fluka or Sigma-Aldrich. All reactions were performed under nitrogen atmosphere in a Synthesis 1 synthesizer (Heidolph Instruments) equipped with PFA reaction vessels. LCMS analyses were done with a Bruker Esquire2000 using ESI in positive mode. ^{1}H NMR spectra were recorded on a Bruker AM 360 (360 MHz) spectrometer. IR spectra were recorded on a Jasco FT/IR 410 spectrometer. Micro analyses were performed by Beetz, Mikroanalytisches Laboratorium, Kronach, Germany.

3.1.1. Azidomethyl polystyrene (4).[13] Merrifield resin (1.0 g, 2.0 mmol/g) was agitated in a PFA vessel with NaN_3 (0.65 g, 10 mmol) in DMSO (10 mL) at 70 °C for 48 h. After being cooled to room temperature, the suspension was filtered through the vessel frit and the resin was rinsed alternately with MeOH (5×20 mL) and CH_2Cl_2 (5×20 mL) and dried in vacuo to give **4** as a colorless resin. FTIR (KBr-pellet) 2096 cm^{-1}.

3.1.2. Triazolymethyl acrylate (TMA) resin (5). Azidomethyl polystyrene (**4**) was agitated in a PFA vessel with proparyl acrylate[14] (1.1 g; 10 mmol), CuI (7.6 mg; 0.04 mmol) DIPEA (1.0 mL, 7.7 mmol) in DMF (4 mL) and THF (4 mL) at 35 °C. The progression of the reaction was monitored by IR spectroscopy. After disappearance of the signal at 2096 cm^{-1}, the suspension was filtered through the vessel frit and the resin was rinsed alternately with pyridine (5×20 mL), MeOH (5×20 mL) and CH_2Cl_2 (5×20 mL) and dried in vacuo to give **5** as a light brown resin. Combustion analysis of nitrogen indicated a loading of 1.63 mmol/g.

3.1.3. Benzyl-(2,6-dichlorobenzyl)-methylamine (7a). TMA resin (**5**) (100 mg) was agitated with benzylmethylamine (200 mg, 1.65 mmol) in DMF (2 mL) for 16 h at ambient temperature. The suspension was filtered through the vessel frit and the resin was rinsed alternately with MeOH (5×5 mL) and CH_2Cl_2 (5×5 mL) and dried in vacuo. The resulting resin **6a** was treated with a solution of 2,6-dichlorobenzyl bromide (200 mg, 0.83 mmol) in DMSO (4 mL) and agitated for 16 h at ambient temperature. After being filtered, the resin was washed with MeOH (3×5 mL) and CH_2Cl_2 (3×5 mL) and TEA (0.2 mL, 14 mmol) in DMF

Table 1. Parallel synthesis using TMA resin



Compound	HX=	R=	Yield[a] (%) (purity, %)[b]	Ref.[6] yield (%) (purity, %)
7b	Tetrahydro-isochinoline	Allyl	82 (95)	88 (>90)
7c	Tetrahydro-isochinoline	*p*-Nitrobenzyl	77 (92)	63 (>90)
7d	Tetrahydro-isochinoline	CH_2CO_2Me	75 (92)	73[c] (>90)
7e	N-Phenyl-piperazine	Allyl	79 (93)	75 (>90)
7f	N-Phenyl-piperazine	*p*-Nitrobenzyl	53 (91)	47 (>90)
8	N-Phenyl-piperazine	CH_2CO_2Me	62[d] (96)	—

[a] Calculated on the loading of **5** (1.63 mmol/g).
[b] Determined by LCMS using MeOH/aq. 0.1 N HCO_2H gradient system on RP-18 material and ESI-ion trap mass spectrometry.
[c] Results for ethyl ester derivative.
[d] Yield of the brominated phenylpiperazine **8**.

(2 mL) was added. Stirring at ambient temperature for 6 h was followed by filtration of the solvent, whereas the resin was washed with DMF (2 mL) and CH_2Cl_2 (3×5 mL). The combined filtrates were washed with a saturated solution of $NaHCO_3$ and evaporated to dryness to give 32 mg (68%) of **7a** as a colorless solid. ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm)=2.18 (s, 3H), 3.62 (s, 2H), 3.84 (s, 2H), 7.13 (t, 1H), 7.21–7.36 (m, 7H); ESI-MS (*m*/*z*) 276 ($M+1^+$).

3.1.4. Parallel synthesis of tertiary amines (7b–f) and (8). Tertiary amines **7b–f** and **8** were synthesized following the procedure described for **7a** starting in each case from 100 mg of **5**. Yields are given in Table 1. Compounds were characterized by LCMS analysis using Agilent Zorbax Eclipse© XDB-C8 (5 μm) and MeOH/0.1 N aq. formic acid gradient system (50:50 to 95/5) in combination with ES ionization and ion trap detection in positive mode and by ^{1}H NMR as follows.

N-Allyl-1,2,3,4-tetrahydroisochinoline (**7b**). ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm)=2.75 (t, 2H), 2.91 (t, 2H), 3.18 (d, 2H), 3.63 (s, 2H), 5.19 (d, 1H), 5.26 (d, 1H), 5.90–6.01 (m, 1H), 6.99–7.13 (m, 4H); ESI-MS (*m*/*z*) 203 ($M+1^+$).

2-(4-Nitrobenzyl)-1,2,3,4-tetrahydroisochinoline (**7c**). ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm)=2.77 (t, 2H), 2.92 (t, 2H), 3.65 (s, 2H), 3.78 (s, 2H), 6.96–7.15 (m, 4H), 7.56–7.61 (m, 2H), 8.17–8.21 (m, 2H); ESI-MS (*m*/*z*) 269 ($M+1^+$).

Methyl-(1,2,3,4-tetrahydroisochinolin-2-yl)-acetate (**7d**). ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm)=2.87–2.97 (m, 4H), 3.43 (s, 2H), 3.75 (s, 3H), 3.80 (s, 2H), 6.98–7.14 (m, 4H); ESI-MS (*m*/*z*) 206 ($M+1^+$).

1-Allyl-4-phenylpiperazine (**7e**). ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm)=2.59–2.63 (m, 4H), 3.06, (d, 2H), 3.19–3.23 (m, 4H), 5.15–5.26 (m, 2H), 5.84–5.96 (m, 1H), 6.82–6.94 (m, 3H), 7.22–7.28 (m, 2H); ESI-MS (*m*/*z*) 203 ($M+1^+$).

1-(4-Nitrobenzyl)-4-phenylpiperazine (**7f**). ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm)=2.60–2.65 (m, 4H), 3.19–3.24 (m, 4H), 3.66 (s, 2H), 6.84–6.95 (m, 3H), 7.24–7.29 (m, 2H), 7.53–7.57 (m, 2H), 8.17–8.22 (m, 2H); ESI-MS (*m*/*z*) 298 ($M+1^+$).

Methyl (4-(4-bromophenyl)-piperazin-1-yl)-acetate (**8**). ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm)=2.73 (t, 4H), 3.21 (t, 4H), 3.29 (s, 2H), 3.74 (s, 3H), 6.76–6.81 (m, 2H), 7.31–7.36 (m, 2H); ESI-MS (*m*/*z*) 313, 315 ($M+1^+$).

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High loading polymer reagents based on polycationic Ultraresins. Polymer-supported reductions and oxidations with increased efficiency

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Abstract—Ultraresin **1** was prepared from highly branched polyethylene imine (M_n=10,000) via reductive cross-linking with terephthaldialdehyde. Following quaternization with methyl iodide, the polycationic Ultraresin **2**, with iodide as a counterion, was obtained. These novel resins combine low swelling with high mechanical stability. By anion exchange polycationic Ultraresins carrying borohydride (**3**) and periodate (**22**) were generated and were investigated as very high loading polymer reagents. Ultra-borohydride resin **3** had a reducing activity of up to 12 mmol/g depending on the substrate. It proved successful in diverse reductions including those of aldehydes, ketones, and nitroolefines. The resin was employed in the reductive amination of aldehydes with an excess of amines, which were removed by the use of a scavenger resin. Periodate resin **22** was obtained with an active loading of up to 5.4 mmol/g and was employed in oxidations of sulfides, diols, hydroquinones, and hydrazines.

1. Introduction

Complex organic molecules can be constructed in homogeneous solution, however, for diversity-oriented purposes it is often advantageous to employ a multiple phase system which greatly facilitates isolation and separation procedures as well as the removal of excess reagents and the completion of reactions. Solid phase synthesis is the most widely applied example for multiple phase systems in combinatorial chemistry, possessing significant advantages in comparison to homogeneous single phase synthesis.

Synthesis in solution, however, possesses indisputable advantages in respect to the versatility of applicable reactions, the ease of analytical monitoring, and the accumulated knowledge of synthetic protocols. Thus, an ideal synthetic strategy would combine these merits with the advantages of solid-phase synthesis protocols, such as the possibility of using reagents in large excess and of removing them by filtration. This combination is realized in polymer-assisted solution phase (PASP) synthesis either by using scavenger resins or by the implementation of polymer reagents.[1–4] Polymer reagents can be used in excess and removed by filtration, the products can be easily analyzed and further transformed in solution. They are especially suitable for automated parallel synthesis. They allow preparation of complex libraries by multi-step syntheses in solution, they can be utilized in automated and in flow-through systems, finally they can be employed to transform single compounds as well as complex mixtures obtained by split-mix combinatorial synthesis.

Many advanced polymer reagents have been developed for demanding reactions over recent years. One significant limitation of current polymer reagents, however, remains their relatively low loading (0.5–2 mmol/g for most commercial reagents). Due to their high price, polymer reagents are not very economical confining the method to small scale or microchemistry applications. High loading and economically produced resins would increase the efficiency and the atom economy[5] of polymer-supported methods considerably. In addition, in high loading and low swelling reagents the concentration of the polymer-supported reactants is enhanced, thus reducing the amount of solvents and of polymer backbone employed. Higher concentrations of the supported reactants should accelerate reaction rates and increase yields.

The first, but still important polymer reagents, were based on anion exchange resins loaded with anionic reagents. Classical ion exchange based polymer reagents, however,

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suffer from several drawbacks. Standard anion exchange resins are obtained from cross-linked chloromethyl polystyrene by nucleophilic substitution with trimethylamine.[6] The maximum loading of such resins is at first limited by the weight of the polymer backbone. If the chloromethyl groups are generated in the resin under Friedel–Crafts conditions, the maximum loading is further restricted by Lewis-acid catalyzed methylene cross-linking of chloromethyl groups within the resin.

Furthermore, the chemical lability of the ammonium salt has to be considered. Typically anion exchange resins display the fishy odor of released trimethylamine. Thus for several reasons, the molar activity of the supported reagents can decrease considerably during storage and can be significantly lower than the original loading as specified by the supplier. Another general problem of polystyrene is its chemcial lability under strong Lewis acidic and oxidative conditions that have been documented thoroughly.[7]

In principle, the maximum loading of carrier materials is obtained in polymers constructed from low molecular weight functional monomers such as polyvinylalcohol, polyethylene imine, or polyvinylamine. Realizing this fact, polyethylene imine (PEI) was selected as the starting material for very high loading polymer supports (Ultraresins).[8]

2. Results and discussion

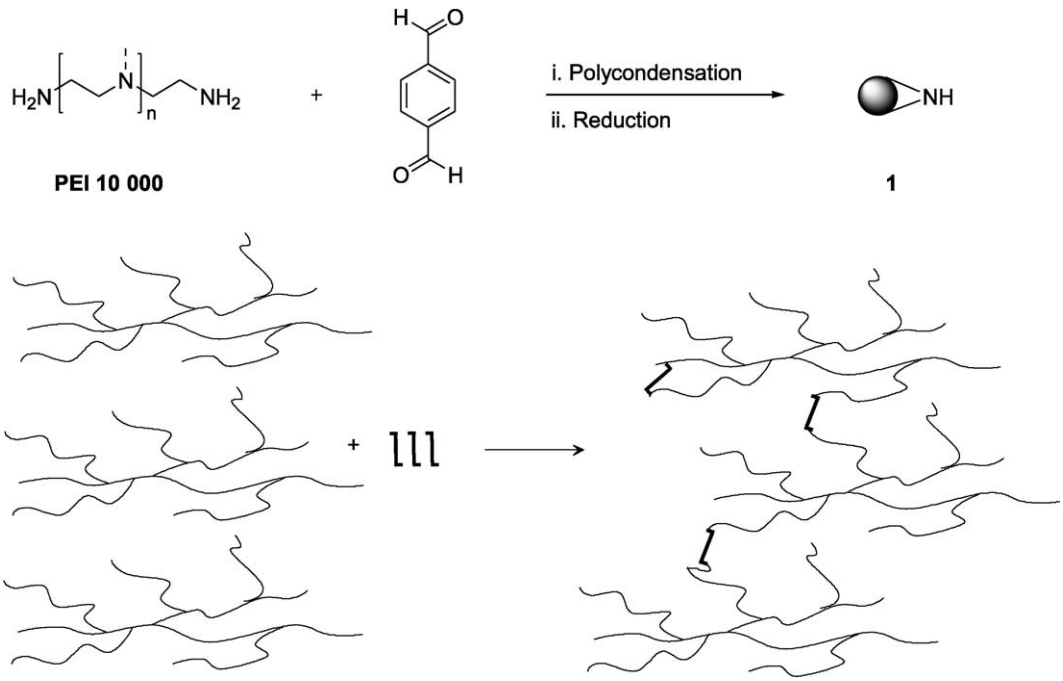
In a recent publication, a collection of Ultraresins with varied cross-linking were constructed from various PEIs and were investigated in respect to their swelling volumes, mobility and synthetic accessibility.[9] Ultraresins constructed from short, prepondarably linear, PEIs were ideally suited for solid phase organic synthesis of peptides and heterocycles.[8] Low-crosslinked Ultraresins displayed large swelling volumes, unless they were chemically derivatized with a linker moiety. Especially when swollen in aqueous hydrochloric acid, the free-amine resins had a swelling volume of up to >100 ml/g resin. This enormous swelling was attributed to the presence of multiple protonated amines. Such very strongly extending resins were not desirable for use as polycationic reagents, as they required larger reaction volumes and led to reduced concentrations.

Thus for use as polycationic polymer reagents, Ultraresins had to be developed with reduced swelling yet retain good accessibility. The swelling of polycationic Ultraresins could be reduced by increasing the amount of the cross-linker. As an undesired side-effect, high cross-linking ratios would however reduce the loading. Thus, as an alternative, highly branched PEI was investigated as the starting material for very high loading resins.

Therefore, polycationic Ultrresins were prepared starting from large, statistically branched polyethyleneimine (M_n=10,000; M_w=25,000). This material contained a mixture of 38% tertiary, 24% secondary, and 38% primary amines and was reacted with terephthalic dialdehyde as the cross-linker to yield the Ultraresin **1**. The cross-linker ratio was 42:1 per equivalent of the starting PEI (Scheme 1).

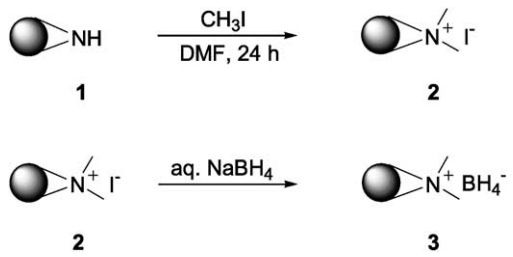
Following the reported condensation–reduction sequence, stacked sieves with calibrated mesh size were used to obtain a size distribution of 90–180 mesh (80–170 μm) or >180 mesh (<80 μm), respectively (Fig. 1).

Quaternization of the secondary and tertiary amines within the resin network of **1** was attained by alkylation with methyl iodide at rt (Scheme 2). The conversion was assessed by elemental analysis of iodine in the washed and dried resin sample yielding an iodine content of 47.5%. The



Scheme 1. Synthesis of Ultraresin **1** from highly branched polyethylene imine yielding a high-loading, low-swelling, and mechanically stable support material.

Figure 1. Polycationic Ultraresin loaded with 12 mmol/g borohydride (effective activity with 4-nitroaldehyde, rt, 16 h). The resin was sieved to a particle size of 80–170 μm (90–180 mesh). The reaction vessel to the left contains 1 mequiv. borohydride resin (82 mg).



Scheme 2. Preparation of polycationic Ultraresin **2** in the iodide form. Loading of polycationic Ultraresin **2** with borohydride anions yielded the reducing resin **3** with up to 12.0 mmol/g reductive activity.

obtained polycationic Ultraresins **2** displayed unique swelling properties, as well as chemical and mechanical stability. The synthetic protocols allowed the preparation of 50 g of **2** without difficulties.

As the first polymer reagent based on the polycationic Ultraresin, the borohydride resin **3** was generated by the exchange of iodide anions from **2**. The swelling volume of **3** was considerably reduced compared to the Ultraresins based on short PEIs. The maximum swelling volume was found in methanol with 10.7 ml/g. The swelling volume was essentially constant when switching from DCM to water. The swelling factor (volume of swollen resin / volume of dry resin) was below 2 for most solvents (Table 1).

Table 1. Swelling properties of borohydride resin **3**

Solvent	Resin volume (ml/g)	Swelling factor
none (dry resin)	5	1
Methanol	10.7	2.14
DCM	8.2	1.64
Water	8.8	1.76
THF	6.0	1.2
DMF	7.5	1.5
Toluene	7.0	1.4

Polystyrene-supported borohydride is one of the classical polymer reagents. Introduced in 1961,[10] it has been employed broadly in polymer-assisted solution phase synthesis in recent years.[11,12] Standard resins are supplied with nominal loadings of ca. 3 mmol/g. Before using these resins in synthesis, it is usually advisable to determine the actual reductive activity in a standardized test reaction, as the active loading can be significantly lower than reported.

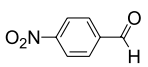
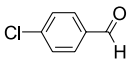
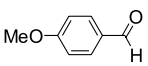
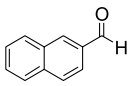
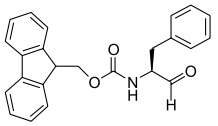
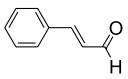
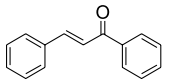
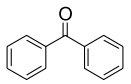
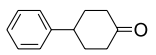
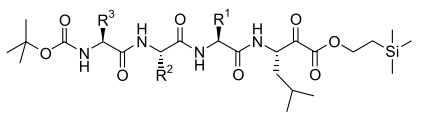
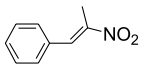
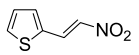
The reduction of 4-nitrobenzaldehyde was selected as a test reaction for activity determination and indicated a reducing activity of 8.0 mmol/g for resin **3** after 0.5 h (1 equiv., Table 2). By increasing the reaction time to 16 h the reducing activity of resin **3** was even 12 mmol/g. Subsequently, a selection of aromatic and aliphatic aldehydes, activated carboxylic acids, aromatic and aliphatic ketones, and α,β-unsaturated nitro-olefines were subjected to polymer-supported reduction with reagent **3** (Scheme 3). As the reaction rates depended strongly on the substrates, reaction times and reagent excess were adapted in order to achieve complete consumption of the starting materials (Table 2). The products were analyzed for purity by HPLC (214 nm) and by NMR-spectroscopy. Aldehydes reacted much faster than ketones and were reduced selectively in the presence of a double bond in α-β-position. On the contrary the double bonds in nitroolefines were reduced without affecting the nitro group. An α-ketoamide-containing fully protected tetrapeptide[13] was reduced to the norstatine product **13** bearing four aliphatic side chains. The products were obtained in good to excellent yields with only few equivalents excess (Table 2), showing that the superior loading of the Ultraresin **3** could be fully exploited in polymer-assisted transformations.

Reductive amination was investigated by treatment of an excess of the amine (1.3 equiv.) with an aldehyde followed by addition of polycationic borohydride resin **3** (Scheme 3, Table 3). A small excess of resin (2 equiv.) sufficed to furnish clean products in good yields, that were obtained by scavenging of the amine excess. Following to the reaction the excess of the primary amine was removed by scavenging with a polymer-supported aromatic aldehyde. Currently, reductions of alternative substrates with resin **3** are under investigation including amides, nitriles, nitro compounds, and azides.

The next target of research was the preparation of an oxidizing polymer. Polycationic Ultraresins such as **2** could be especially valuable as carriers for oxidative species as the oxidative sensitivity of polystyrene has been documented well and has limited the extended use of polymer-supported oxidants so far. Recently, this group during the synthesis of supported IBX-resin has reported on the partial oxidation of polystyrene by treatment with tetrabutylammonium oxone at 80 °C[14] and by oxoammonium salts.[15]

Before the loading of resin **2** with oxidative species, the iodide anions had to be exchanged for chloride in order to avoid the formation of iodine by oxidation. For this purpose, **2** was washed with aqueous HCl (1 M) until no residual iodide could be detected in the washing solution by the addition of hydrogen peroxide. The resin **21** obtained was then treated with sodium periodate solution, washed, and dried yielding periodate resin **22** (Scheme 4). The presence of periodate was assessed qualitatively by IR and by reaction of a resin sample with sulfuric acid and

Table 2. Reactions employing reducing Ultraresin **3**

Product	Starting material	Equivalents[a]	Time (h)	Yield (%)	Purity (%)
4		1	0.5	98	100
5		1.3	1	98	100
6		2	4	100	99
7		1	2	100	99
8		2	3	95	90
9		1.6	4	100	99
10		4	16	100[b]	81
11		4	16	98	100
12		4	16	100[c]	100
13		8	16	65[c]	90
14		2	2	77[d]	100
15		2	2	52[d]	93

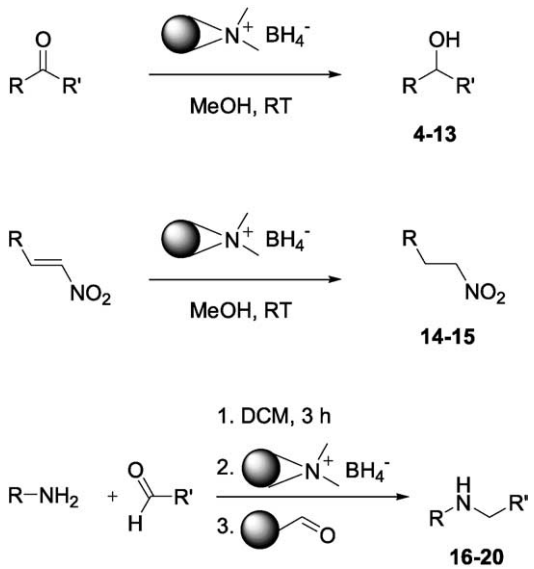
As determined by ^{1}H NMR-spectroscopy.
[a] Calculated for an effective loading of 8 mmol/g.
[b] 19% reduced to the corresponding saturated alcohol.
[c] Obtained as mixture of 2 diastereomers.
[d] Reduction to the saturated nitroalkane.

o-phenylenediamine leading to a red precipitate.[16] The oxidative activity of resin **22** was measured by the partial oxidation of an excess of 1,4-hydroquinone to quinone **25** as quantified by NMR-spectroscopy. The active loading in this reaction was 5.4 mmol/g resin. This value exceed the maximum loading obtainable with the periodate ion. The supported periodate ion thus can be assumed to perform a two step oxidation via the intermediary iodate (Scheme 5, Table 4).

3. Conclusions

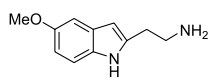
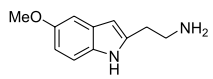
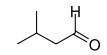
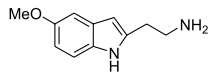
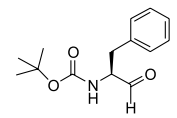
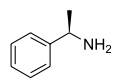
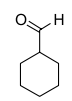
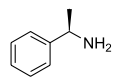
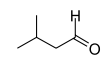
The original Ultraresin concept was successfully extended towards the generation of polycationic Ultraresins **2** which could be used as polymer reagents in a broad selection of oxidation and reduction reactions. By selecting highly branched high molecular weight PEI as starting material a polymer was obtained that was chemically and mechanically stable and was tailored to swelling volumes well-suited for use as anion-carrying polymer reagents. The resin was produced with narrow particle size distribution and low swelling factors in most solvents. Thus, it might be useful in continuous flow reactors that allow operation with low back pressures.

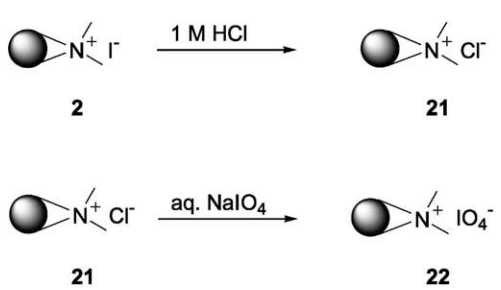
The economic preparation of the support is noteworthy. All starting materials are inexpensive industrial bulk products. Resin production can be up-scaled with ease and does not require the addition of emulgators and multiple phase



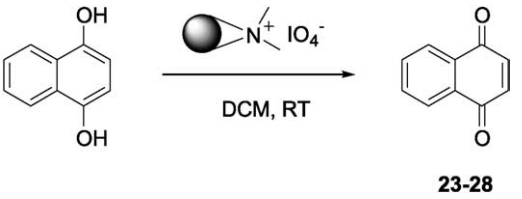
Scheme 3. The borohydride resin **3** was employed for the conversion of various aldehydes, ketones and nitro-olefines and for reductive amination yielding products **4**-**20**.

Table 3. Reductive aminations with resins **3**: following to 3 h, 2 equiv. of borohydride resin **3** was used and reacted 16 h at room temperature. Scavenging of the primary amine with 10 equiv. of formyl-PS (0.57 mmol/g) for 6 h at 40 °C

Product	Amine	Aldehyde	Yield (%)	Purity (%)
16			85	99
17			83	91
18			94	94
19			92	90
20			72	85



Scheme 4. The oxidizing periodate resin **22** was obtained via the chloride-loaded polycationic Ultraresin **21**.



Scheme 5. The oxidizing resin **22** was employed for the conversion of various sulfides, hydroquinones, diols, and hydrazines yielding products **23**-**28**.

systems as for polystyrenes. In addition, the obtained Ultraresins are chemically and mechanically robust; the lability towards oxidants and Lewis acids is strongly reduced in comparison with standard support materials.

The demonstrated applications of the novel resin include a reducing borohydride resin **3** which was generated with an active loading of up to 12.0 mmol/g, significantly higher than for conventional borohydride exchange resins. The resin performed successfully in reductions of aldehydes, ketones and in reductive aminations. The polymer-supported periodate resin was obtained with an active loading of up to 5.4 mmol/g and very efficient in the oxidation of sulfides, hydroquinones, hydrazines, and in periodate-cleavage of diols.

As found for applications in solid phase synthesis, the accessibility of the novel Ultraresin-based polymer reagents **3** and **22**—despite of their high loading—remain fully accessible for soluble reactants. This is shown not only by the determined activity of the reagents but as well by the efficiency in less-favored transformations. Therefore, Ultraresin-based polymer reagents should be a viable alternative to current support materials.

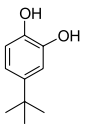
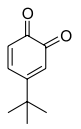
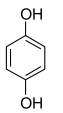
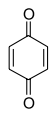
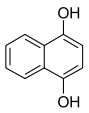
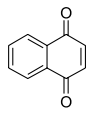
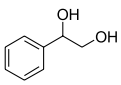
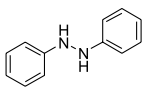
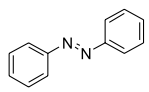
4. Experimental

4.1. General procedures

Polyethylene imine (PEI) (M_n=10,000, M_w=25,000) was obtained from Sigma Aldrich. Solvents were purchased in HPLC grade or freshly distilled before use. Formyl polystyrene resin was a gift from Merck Biosciences, Läufelfingen, Switzerland. All reactions were carried out in glass tubes. The NMR measurements were conducted on a Bruker Avance 400 MHz spectrometer. The IR spectra were measured on a Bruker Vector 22 FT-IR spectrometer employing a split-pea ATR unit. For HPLC analysis a Beckman Gold system was used with a diode array detector and an analytic reversed phase column (Nucleosil 100 C-18, 5 μm, 2×250 mm, Fa. Grom, Herrenberg) operated with acetonitril–water mixtures containing 0.1% trifluoroacetic acid.

4.1.1. Synthesis of ultra resin (1). PEI 10,000 (21 g) was dissolved in THF (150 ml) in a 500 ml round-bottom flask. Terephthalic dialdehyde (11.9 g, 0.088 mol, dissolved in 125 ml THF) was added rapidly to the stirred solution. The reaction mixture solidified spontaneously and was shaken for one hour. The cross-linked polymer was washed with THF, crushed, and suspended again in a mixture of THF

Table 4. Oxidations employing the periodate resin **22**

Product	Starting material	Product	Equivalents[a]	Time (h)	Yield (%)	Purity (%)
23			1.63	1[b]	94	97
24			2.2	0.5[c]	100	100
25			1.4	1[d]	100	99
26			2.1	0.33[d]	100	93
27			2.1	10[d]	91	93
28			2.1	4[c]	88	85

[a] Calculated for an effective loading of 3 mmol/g.
[b] In MeOH.
[c] In DCM.
[d] In DCM/MeOH 2:1.

(300 ml) and methanol (150 ml). Sodium borohydride (6.7 g, 0.17 mol) was added and the mixture was stirred for 2 h. To remove excess sodium borohydride the polymer was transformed in its hydrochloride form by treatment with 1 N hydrochloric acid for one hour. The polymer was passed through a frit, washed with water and was stirred in 2 N sodium hydroxide solution for 15 min to convert the polymer to the free amine form **1**. After washing with water, THF and dichloromethane (DCM) the polymer was dried in vacuo at 60 °C for 5 h. The polymer was sieved to get particles of 90–180 mesh (80–170 μm). Yield 28 g (90%) of **1**. Elemental analysis C 53.1%, H 9.9%, N 21.1%, Cl 0.1%. Loading of amine 15 mmol/g. FT-ATR-IR: 798, 1114, 1460, 1508, 2817, 2937, 3220.

4.1.2. Synthesis of polycationic Ultraresin 2. Ultraresin **1** (20 g) was swollen in N,N-Dimethylformamide (DMF) (500 ml) and methyl iodide (426 g, 3 mol) was added. After shaking for 24 h at room temperature, the quaternized polymer was transferred to frit (glass filter), washed with DMF and DCM and dried in vacuo at 60 °C for 5 h. Yield: 42.7 g of a pale yellow polymer. Elemental analysis C 31.3%, H 5.8%, N 9.2%, I 47.5%. FT-ATR-IR: 1460, 1612, 2784, 2949, 3402.

4.1.3. Loading of polycationic Ultraresin 2 with borohydride anions yielding reducing resin 3. Quaternized ultra resin (**2**, 32 g) was swollen in water and a 1 M aqueous solution of sodium borohydride was poured over the resin for 15 min. After repeating this procedure for two times the resin was washed with distilled water until free from excess sodium borohydride. The resin was dried in vacuo at 80 °C for 5 h to yield 16 g of borohydride exchange Ultraresin **3** as a white polymer. FT-ATR-IR: 1070, 1456, 1578, 2223, 2814, 2939. The polymer was stored at 4 °C and the hydride content was stable for 3 months.

4.1.4. Reduction of carbonyl compounds and nitroolefines to products 4–15. The following reduction of 4-chlorobenzaldehyde is representative for the reduction of carbonyl compounds and nitroalkenes. 4-Chlorobenzaldehyde (23.6 mg, 0.168 mmol) was dissolved in 2 ml methanol. After adding 28 mg of borohydride Ultra resin **3** (0.22 mmol with an effective loading 8 mmol/g) the reaction mixture was shaken for one hour at room temperature. The completion of the reaction was indicated by thin layer chromatography. After adding 4 ml of DCM the resin was removed by filtration and washed for three times with 2 ml of DCM. The solvent was removed by evaporation to get pure 4-chlorobenzylalcohol. The purity was checked by ^{1}H and ^{13}C NMR-spectroscopy and HPLC analysis (detection wavelength 214 nm).

4.2. General procedure for the reductive amination to amines 16–20

5-Methoxytryptamine (25.2 mg, 0.133 mmol) and cyclohexanecarboxyaldehyde (11.4 mg, 0.102 mmol) were dissolved in 2 ml methanol. After shaking for 3 h at room temperature borohydride Ultra resin **3** (51 mg, 2 equiv.) was added and the reaction mixture was shaken for 16 h at room temperature. The resin was removed by filtration, washed

three times with 2 ml of DCM. To remove excess amine a formyl polystyrene resin (0.57 mmol/g, 10 equiv.) was used. The mixture was shaken for 6 h at 40 °C. After filtration of the resin and washing with 15 ml of DCM the solvent was evaporated to get the secondary amine **16**. Purity was checked by ^{1}H and ^{13}C NMR-spectroscopy and HPLC analysis (detection wavelength 214 nm).

4.2.1. Loading of polycationic Ultraresin 2 with chloride anions yielding resin 21. Quaternized ultra resin (**2**, 2 g) was swollen in water and a 1 M aqueous solution of hydrochloric acid was added then the reaction mixture was shaken for 30 min at room temperature. After repeating this procedure for five times the resin was washed with distilled water until free from excess hydrochloric acid. The resin was dried in vacuo overnight to yield 1.8 g of chloride exchange Ultra resin **21** as a white polymer. FT-ATR-IR: 1021, 1472, 1638, 2821, 2969, 3376.

4.2.2. Loading of polycationic Ultraresin 21 with periodate anions yielding oxidizing resin 22. Quaternized ultra resin (**21**, 1.8 g) was swollen in water and an aqueous solution of sodium periodate (5 equiv.) was added. The reaction mixture was shaken for one hour at room temperature. After repeating this procedure for three times the resin was washed with distilled water until free from excess periodate and finally three times with acetone. The resin was dried in vacuo overnight to yield 1.7 g of periodate exchange Ultra resin **22** as a white polymer. FT-ATR-IR: 780, 841, 1473, 1654, 3022.

4.3. General procedure for the oxidation of diols, sulfides, quinols and catechols to products 23–28

The following oxidation of thioanisole to **23** is representative for the oxidation of diols, sulfides, quinols and catechols. Thioanisole (15 mg, 0.12 mmol) was dissolved in 1 ml methanol. After adding 65 mg of periodate resin (effective loading 3 mmol/g) the reaction mixture was shaken for 1 h at room temperature. The completion of the reaction was indicated by thin layer chromatography. The resin was filtered off and washed for three times with 2 ml of DCM and two times with methanol, all solvent was removed by evaporation to furnish **23**. The purity was checked by ^{1}H and ^{13}C NMR-spectroscopy and HPLC analysis (detection wavelength 214 nm).

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Dendritic polyglycerol as a high-loading support for parallel multistep synthesis of GABA lactam analogues

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Abstract—A general route to 4-substituted azolidin-2-ones (GABA lactam analogues) on a soluble high-loading polyglycerol support has been developed and optimized. These biologically interesting compounds (anticonvulsive drugs) can be synthesized in three steps commencing from a polyglycerol supported (diethylphosphono)acetic acid and a carbonyl compound. The key features of this parallel approach are the cyclative cleavage and simple separation techniques (i.e., dialysis).

1. Introduction

High-loading soluble polymeric supports for organic synthesis can overcome some of the problems associated with solid phase supports, such as heterogeneous reaction conditions and therefore disadvantageous kinetics, low loading capacities, exacerbated analysis, and problematic mechanical stability.[1] Soluble supports feature homogeneous reaction conditions and enable the application of standard analytical techniques (TLC, IR, NMR, MALDI-TOF, etc.) as well as the orthogonal[2] use of insoluble reagents. One drawback of soluble polymeric supports is the fact that there is no generally applicable separation technique as for solid phase supports. However, a variety of simple separation methods exists, such as conventional precipitation, liquid–liquid extraction, and filtration over silica-gel, or techniques which separate by molecular size such as dialysis, ultrafiltration, and size exclusion chromatography (SEC).[3] Several recent reviews on soluble polymeric supports demonstrate their general use and application in organic synthesis including simple separation techniques.[1,3–9]

Some of the most commonly used linear soluble polymeric supports have the disadvantage of either very low loading capacities (e.g., monomethylated poly(ethylene glycol), 5000 g mol^{-1} (MPEG 5000), 0.2 mmol g^{-1}, only one reactive group per polymer molecule) or problematic polymer characteristics, such as solubility and chemical stability (e.g., poly(vinyl alcohol) (PVA), 22.7 mmol g^{-1}, reactive group on every monomer unit).[1,5] In some cases, these disadvantages can be overcome by the use of branched polymer architectures.[1,10] An extreme in terms of tree-like branching are the perfectly branched dendrimers.[11] These well-defined macromolecules are soluble in many organic solvents (depending on their shell functionalities) and possess a maximum capacity of easily accessible functional groups in their periphery. Dendrimers as supports for organic synthesis, mainly catalysis, have been frequently used.[9,10,12–14] However, the general drawback of any dendrimer is the tedious and expensive multistep preparation of higher generations.[1,15] Yet another problem of high-generation dendrimers appears to be steric hindrance and site–site interaction at the outer functional shell. These problems might be overcome by using randomly branched polymer structures as supports.[1,13,15] In contrast to dendrimers, hyperbranched polymers are easily available in one reaction step in large quantities.[16] They contain dendritic, linear and terminal monomer units in their skeleton and hence can be considered as intermediates between linear polymers (degree of branching: DB=0%) and dendrimers

Keywords: Soluble polymeric support; Hyperbranched polymer; Dendrimer; Azolidinone; Membrane separation; Parallel synthesis.

Abbreviations: abs., absolute; aq., aqueous; DB, degree of branching; DBU, 1,8-diazabicyclo[5.4.0]undec-7-en; DCC, dicyclohexyl carbodiimide; DMAP, *N*,*N*-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; DVB, *o*/*p*-divinylbenzene; GABA, γ-aminobutyric acid; GPC, gel permeation chromatography; IR, infrared; LDA, lithium diisopropylamide; LiHMDS, lithium hexamethyldisilazide; MALDITOF, matrix assisted LASER desorption ionization time of flight; MPEG, monomethylated poly(ethylene glycol); MWCO, molecular weight cut-off; NMR, nuclear magnetic resonance; p.a., pro analysi; PG, polyglycerol; PVA, poly(vinyl alcohol); rt, room-temperature; SEC, size exclusion chromatography; TBAF, tetrabutylammonium fluoride; TBME, *tert*.butyl methyl ether; THF, tetrahydrofurane; TLC, thin layer chromatography; TMG, 1,1,3,3-tetramethyl-guanidine.

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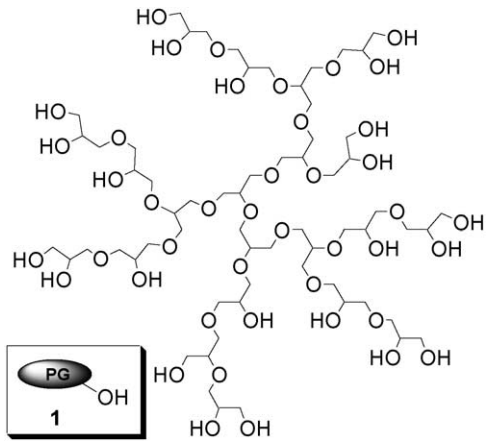


Figure 1. Dendritic polyglycerol support. The depicted polymer structure represents only one possible isomer and a small part of the polyglycerol (M_n=8000 g mol^{-1}) scaffold.

(DB=100%) with an approximate DB between 50 and 75%.[1] In contrast to dendrimers, hyperbranched polymers[17] are polydisperse and the reactive sites will be distributed throughout the molecules, but it has been shown that in some cases the support properties of hyperbranched polymers are very similar to those of analogous dendrimers and thus, structural perfection is not always required.[13,15,18,19] The potential loading capacity of hyperbranched polymers is similarly high as for dendrimers (5–14 mmol g^{-1}) and thus allows for parallel synthesis on the millimole scale.[1]

In previous publications, we have reported on the use of hyperbranched polyglycerol **1** (Fig. 1) as a high loading polymeric support for carbonyl compounds[18] or boronic acids for polymer supported Suzuki-reactions.[20] In both cases, immobilization can be performed using the 'built-in' terminal 1,2-diols and no additional linker was necessary. Other interesting features of this soluble aliphatic polyether support are its high loading capacity (13.5 mmol OH g^{-1}) and easy accessiblity on a kilogram scale.[21,22] In addition, it is soluble in most organic solvents (depending on the OH-group functionalization) and the polyether skeleton is stable under a wide range of reaction conditions. Also, we have demonstrated, that polyglycerol and its derivatives can be separated from low molecular weight compounds by simple separation techniques (e.g., membrane techniques or liquid–liquid-extraction).[1,3,18,20]

In this paper, the use of polyglycerol **1** as a high-loading dendritic support[23] for the multistep synthesis of GABA lactam analogues **4** (GABA=γ-aminobutyric acid) (Fig. 2) is described. The commercial drug GABA-pentin **3** is useful in the therapy of certain cerebral disorders such as certain forms of epilepsy, faintness attacks, hypokinesia, and cranial traumas.[24] Recently, GABA-pentin lactam **4a** was found to be a more effective anticonvulsive than the free amino acid **3**.[25,26] In addition to its anticonvulsive activity compound **4a** shows a neuroprotective effect.[27,28] Thus, it was our goal to develop a parallel approach to some related compounds in order to obtain GABA-lactam-analogues **4** for biological evaluation. Our retrosynthetic analysis traces the lactams back to the carbonyl compound **6** and a polyglycerol supported phosphonic acid **5**. As a key intermediate we wanted to generate a γ-amino ester which should release the desired lactam from the polymeric support selectively upon cyclative cleavage (cf. Scheme 1).

2. Results and discussion

Three synthetic routes to the parent compound GABA-pentin lactam **4a** have been described in the literature.[24,29,30] Both require purification by crystallization, distillation or column chromatography after each step. Our polymer supported approach to GABA-lactam-analogues **4** is outlined in Scheme 1. In this case, only one chromatographic purification is needed. In an initial esterification the polyglycerol support **1** is coupled with (diethylphosphono)acetic acid **7** to provide the Horner–Wadsworth–Emmons reagent **5**, which is then olefinated with the carbonyl compound **6**. Subsequent Michael-addition of nitromethane yields the γ-nitroester **9**. Reduction of the nitro group results in in situ-formation of the corresponding amine **10**, which can split off from the polymeric support by cyclative cleavage to generate the lactam **4**. Ideally, the cyclative cleavage[3] step should be selective, since only those species which have passed every single reaction step of the sequence successfully can be released.

We used the dicyclohexyl carbodiimide (DCC)/*N*,*N*-dimethylaminopyridine (DMAP) coupling as a general esterification method[31] between polyglycerol **1** and carboxylic acids (Table 1). The reaction was carried out in DMF and the formed urea can be removed by filtration and

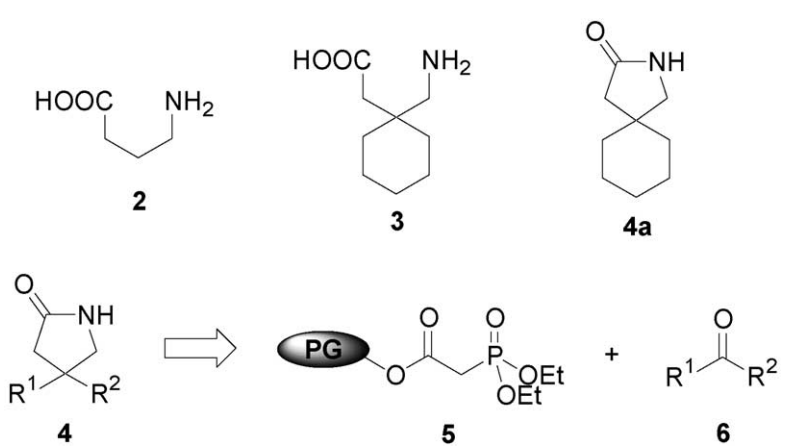
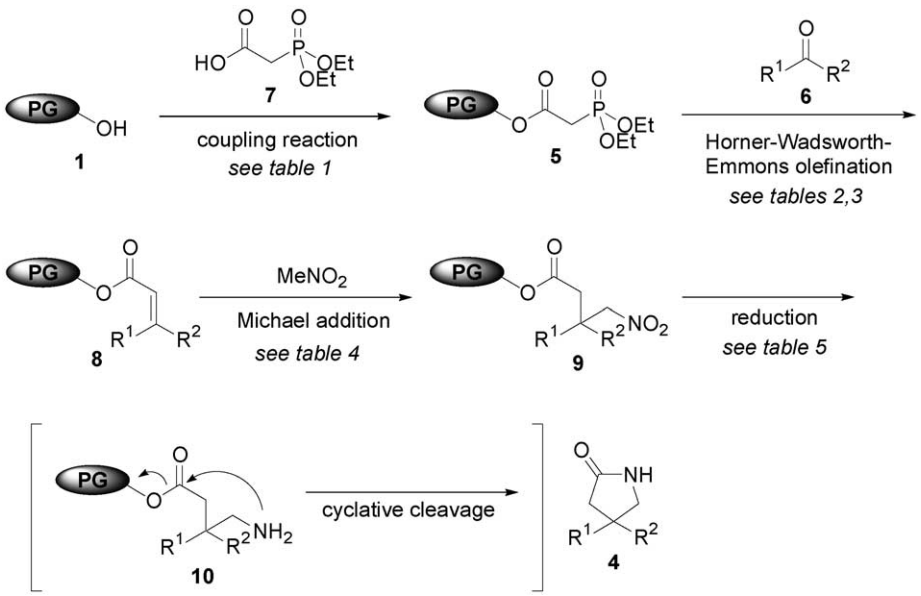
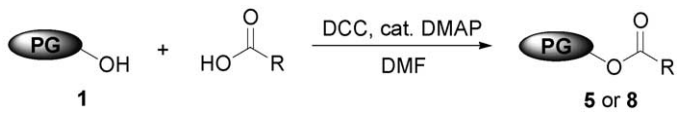


Figure 2. Structures of GABA analogues and retrosynthetic approach to GABA lactam analogues **4**.



Scheme 1. General synthetic route to GABA lactam analogues **4** using polyglycerol **1** as a soluble polymeric support.

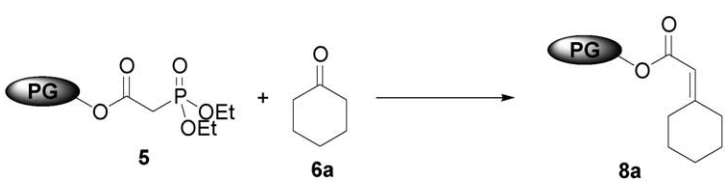
Table 1. Immobilization of carboxylic acids on the polyglycerol support **1** via DCC-couping



Entry	Carboxylic acid	Product	Conversion[a] (%)	Yield[b]
1	(Diethylphosphono) acetic acid	**5**	91	83%
2	Cinnamic acid	**8f**	38	Quant.
3	*p*-Bromo cinnamic acid	**8h**	53	99%
4	*p*-Fluoro cinnamic acid	**8j**	46	80%
5	*p*-Methoxy cinnamic acid	**8k**	39	71%
6	3-(Thien-2-yl)acrylic acid	**8l**	46	79%

Reaction conditions: DCC, DMAP (cat.), DMF, 0 °C→rt, 12 h; workup: filtration and dialysis in $CHCl_3$.
[a] The conversion is the percentage of groups on the polyglycerol support which readily underwent the respective reaction. This value is determined via ^1H NMR (±5%).
[b] Isolated yield based on the ratio of obtained mass to expected mass considering the conversion.

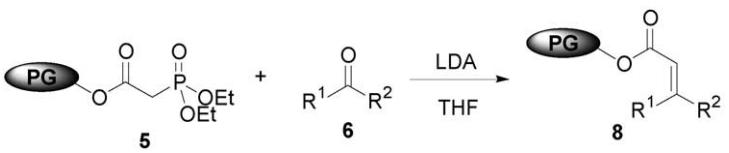
Table 2. Optimization of the Horner–Wadsworth–Emmons olefination between polyglycerol supported (diethylphosphono)acetic acid **5** and cyclohexanone **6a** (R^1/R^2=–$(CH_2)_5$–)



Method	Cyclohexanone **6a** (equiv.)	Base	Conversion[a] (%)	Yield (after extractive workup and dialysis) (%)
A	1	LiHMDS 1.75 equiv.	93	63
B	1	NaH 1.1 equiv.	87	56
	1	LDA 1.1 equiv.	>95	58
	1	LiHMDS 1.1 equiv.	92	65
	1	KO*t*Bu 1.1 equiv.	≈90[b]	69[c]
C	1	LDA 2.3 equiv.	≈90[b]	42%[c]

Reaction conditions: abs. THF, rt, 12 h; workup: quenching (diluted aq. HCl), extraction, dialysis in $CHCl_3$.
[a] via ^1H NMR (±5%).
[b] Estimated from ^{13}C NMR (±10%).
[c] Product contains isomerized byproduct.

Table 3. Multiparallel Horner–Wadsworth–Emmons reactions between the polyglycerol supported (diethylphosphono)acetic acid **5** and different carbonyl compounds **6a-i**



Carbonyl compound	Conversion[a]	Yield (after dialysis)
6a (R^1/R^2=–$(CH_2)_5$–)	90%	Quant.
6b (R^1/R^2=–$(CH_2)_2$–C(H)*t*Bu-$(CH_2)_2$–)	90%	86%
6c (R^1/R^2=–$(CH_2)_2$–C(H)Cl–$(CH_2)_2$–)	65%	82%
6d (R^1/R^2=–$(CH_2)_2$–C(H)Br–$(CH_2)_2$–)	55%	Quant.
6e (R^1/R^2=–$(CH_2)_2$–C(H)OH–$(CH_2)_2$–)	55%	43%
6f (R^1=H, R^2=Ph)	65%	Quant.
6g (R^1=H, R^2=*p*-C_6H_4–Cl)	70%	Quant.
6h (R^1=H, R^2=*p*-C_6H_4–Br)	80%	94%
6i (R^1=H, R^2=*p*-C_6H_4–OH)	s.m.[b]	s.m.[b]

Reaction conditions: abs. THF, LDA (1.1 equiv.), rt, 12 h; workup: hydrolysis, concentration, dialysis in $CHCl_3$.
[a] via ^{1}H NMR (±5%).
[b] Although 2 equiv. base were employed, only starting materials was recovered.

subsequent dialysis. This way, the (diethylphosphono)acetic acid polyglycerylester[32] **5** was obtained in high conversion and yield (Table 1, entry 1).

For the optimization of the Horner–Wadsworth–Emmons olefination between **5** and cyclohexanone **6a** (R^1/R^2=–$(CH_2)_5$–) several bases[33,34] were tried (sodium hydride,[35] lithium diisopropylamide (LDA),[36] lithium hexamethyldisilazide (LiHMDS), potassium *tert*-butylate[37]) in different amounts and different addition sequences, either adding a solution of the base to a solution of the substrates (method B, C) or vice versa (method A) in THF (Table 2). As expected, using method A reversible heterogenization due to polyanion formation occurs. We also observed double bond isomerization using excess base, which is known for cyclohexylideneacetic acid esters.[38,39] In this comparison LDA gave the highest conversions and yields with the lowest amounts of isomerization byproduct and hence was used as the base of choice.

With this optimized reaction conditions in hand several carbonyl compounds were introduced in a multiparallel fashion (ten reactions) (Table 3). This was performed either manually or with a Chemspeed Automated Synthesis Workstation®. Multiparallel workup strategies were also applied. Workup consists of addition of diluted aq. HCl, evaporation (e.g., with a multiparallel evaporator: IR-Dancer®,[40]) and dialysis in a continuous multiparallel dialysis apparatus, on which we have reported recently.[3,18] Exemplarily for the cyclohexylideneacetic acid polyglycerylester **8a** (R^1/R^2=–$(CH_2)_5$–), the efficiency of dialysis for the purification of crude polymer-bound products from low molecular weight compounds is shown in Figure 3.

In some cases, cinnamic acid ester derivatives **8f-l** can also be obtained via direct immoblization of the corresponding cinnamic acid using the DCC/DMAP coupling protocol (see Table 1, entries 2–6). However, the route via Horner–Wadsworth–Emmons–reaction is more flexible and can lead to higher overall conversions. For example, compound **8f** is obtained in 59% conversion with the two step variant, whereas direct immobilization of cinnamic acid only results in 38% conversion (Table 1, entries 1 and 2; Table 3).

Due to steric hindrance and modest activation by the ester groups, Michael-additions at β,β-disubstituted α,β-unsaturated esters are not trivial reactions.[24] Nevertheless, a mild method without large excesses of nitromethane and heating was used because basic nitromethane solutions are potentially explosive. Among the tested bases poor conversion was observed with 1,1,3,3-tetramethyl-guanidine (TMG)[41,42] or 1,8-diazabicyclo[5.4.0]undec-7-en (DBU).[43] However, the reaction can simply be driven to completion using tetrabutylammonium fluoride (TBAF).[29,44] Unexpectedly, we were not able to remove TBAF by means of dialysis in THF, which might be due to micelle formation. Since extraction of this compound is tedious due to its phase-transfer properties, we looked for another simple method to remove this base.[45] As reported recently, polyglycerol derivatives do not interact with crosslinked solid phase resins (e.g., ion exchange resins crosslinked with >5% divinylbenzene (DVB)).[18] Thus, a 1:1-mixture of both anion and cation exchange resins was added, which remove TBAF completely with H_2O-formation. The results of the parallel Michael-addition of the polyglycerol supported α,β-unsaturated esters **8** to the immobilized γ-nitroesters **9** are summarized in Table 4.

Unexpectedly, the key-step, namely the reduction of the nitrogroup to the respective amine and subsequent cyclative cleavage of the desired GABA-lactam-analogue **4**, turned out to be the most difficult step within this reaction sequence. Recently, Köllner and Togni reported on a similar problem reducing an aromatic nitrogroup.[46] A variety of selective reduction methods for aliphatic nitrogroups has been described in the literature[47,48] and tested for this application: H_2/Pd/C, H_2/Raney–Ni, $NaBH_4/NiCl_2{\cdot}6H_2O$, HCOOH/$Pd(OAc)_2$, Zn/HCl, SmI_2, $SnCl_2{\cdot}2H_2O$,[49] cyclohexene/$Pd(OAc)_2$, and $NH_4COO/Pd(OAc)_2$. However, in most cases no product was formed or complex mixtures were observed. The well-known Zn/HCl[50,51] protocol for

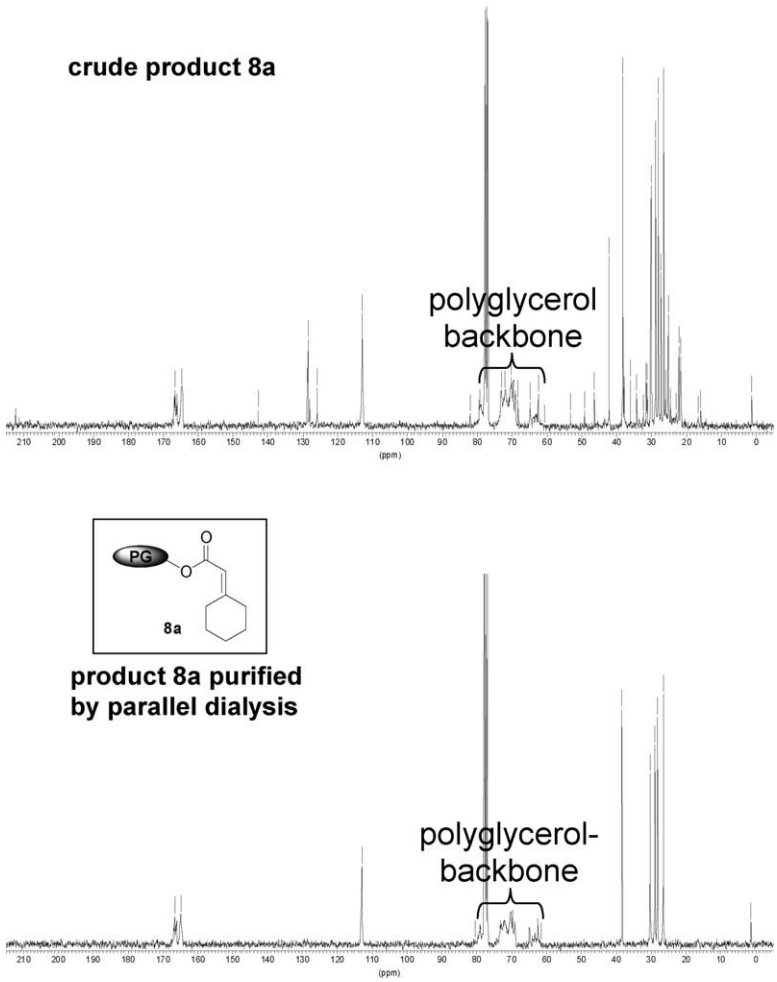
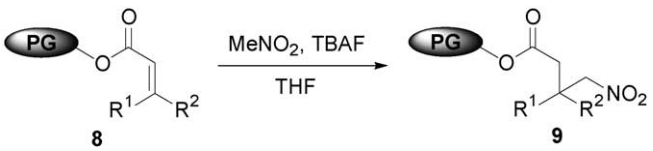


Figure 3. Demonstration of the dialysis efficiency (4 mmol scale): ^{13}C NMR-spectra of cyclohexylideneacetic acid polyglycerylester **8a**. Upper spectrum: crude product, lower spectrum: final product after purification by means of parallel dialysis (24 h) in $CHCl_3$. The signals of the polyether skeleton are labelled as 'polyglycerol backbone'.

the reduction of aromatic nitrogroups, was found to be the most efficient method. Although this final cleavage step occurs only in moderate conversions (Table 5) good overall yields (35–70%) for the four-step synthesis of these cyclic lactams **4** have been realized.

Table 4. Parallel polyglycerol supported Michael-addition



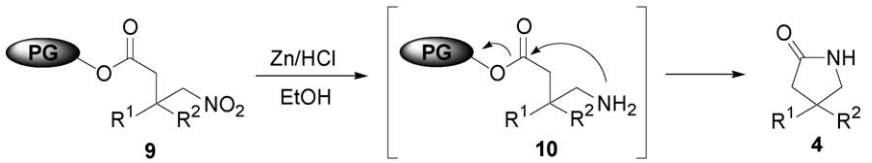
Substrate	Conversion[a]	Yield (%)
8a (R^1/R^2=–$(CH_2)_5$–)	78%	55
8b (R^1/R^2=–$(CH_2)_2$–C(H)*t*Bu-$(CH_2)_2$–)	≈90%	48
8f (R^1=H, R^2=Ph)	Quant.	68
8h (R^1=H, R^2=*p*-C_6H_4–Br)	Quant.	72
8j (R^1=H, R^2=*p*-C_6H_4–F)	Quant.	24
8k (R^1=H, R^2=*p*-C_6H_4–OMe)	Quant.	60
8l (R^1=H, R^2=thien-2-yl)	Quant.	64

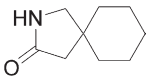
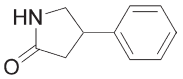
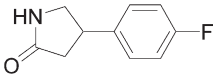
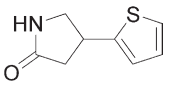
Reaction conditions: nitromethane (4 equiv.), TBAF (2 equiv.), THF, 40 °C, 12 h; workup: combination of anion and cation exchange resin; dialysis in $CHCl_3$.
[a] via ^1H NMR (±5%).

3. Conclusions

In summary, we have shown that dendritic polyglycerols are suitable for parallel multistep synthesis and established the first polymer-supported synthesis for GABA lactam analogues. The polyglycerol support is compatible with many reaction conditions including organometallic reagents and allows for simple purification using membrane techniques as well as the orthogonal use of solid-phase supported reagents (e.g., ion exchange resins). Although dialysis is not yet the optimal method for quantitative retention of the polymeric support (isolated yields are typically between 60 and 100%) it is simple and efficient for the 1–10 mmol scale. It was also demonstrated that polyglycerol can be introduced into automated synthesis using a liquid handler. However, due to the limited library size, no real acceleration was achieved by automation. Despite the last step, we were able to optimize the other reaction steps in a satisfactory way and obtained high overall conversions. In order to estimate the quality of polyglycerol as compared to other supports we are currently investigating this reaction sequence in combination with other polymeric supports. In addition, the GABA lactam analogues obtained are currently undergoing screening for their biological activity.

Table 5. Reduction of the polymer supported γ-nitroesters **9** and concomitant split off of GABA-lactam analogues **4** from the polyglycerol support



Compound		Purification	Overall yield (%)
4a		Filtration over silica gel	58
4b		Column chromatography	70
4f		Filtration over silica gel	36
4j		HPLC	40
4l		HPLC	35[a]

Reaction conditions: Zn/HCl in EtOH, rflx, 1.5–4 h (reaction control via TLC).
[a] Contains an impurity which could not be removed by HPLC.

4. Experimental

4.1. General

Unless declared otherwise, NMR-spectra were obtained on a Bruker ARX 300 at 300 K (^{1}H NMR: 300 MHz, ^{13}C NMR: 75.4 MHz, solvent- or external standards, sample amount: ^{1}H NMR: 10–20 mg, ^{13}C NMR: 60–100 mg). Solvent calibration was performed according to the literature.[52] IR-spectra were obtained on a Bruker Vector22 FT-IR-spectrometer in the range of 4000–500 cm^{-1} (film on KBr). For dialysis, we used the continuous dialysis apparatus which we have recently introduced.[18] Dialysis was performed in benzoylated cellulose dialysis tubes from Sigma-Aldrich (No. D-7884, width: 32 mm, molecular weight cut-off (MWCO) 1000 g mol^{-1}).

Polyglycerol **1** (8000 g mol^{-1}, 13.5 mmol OH groups g^{-1}) was prepared according to the published procedures.[22,53] Other chemicals are commercially available. For water-free procedures, the solvents were dried conventionally. The employed glassware was dried overnight at 105 °C and heated and flushed with N_2 or Ar, respectively, three times just before the reaction. Addition of chemicals was carried out under argon. The acidic ion exchange resin Lewatit™ (Bayer) was washed with methanol before use.

4.2. General procedure for the immobilization of carboxylic acids on the polyglycerol support 1 via DCC/DMAP esterification

This reaction was performed under an inert gas atmosphere and exclusion of water. Polyglycerol **1** (9.4 g, 127 mmol OH-groups, 1 equiv.) was dissolved in abs. DMF (140 ml) upon ultrasonification. During stirring DMAP (1.43 g, 11.0 mmol, 0.09 equiv.) and of the carboxylic acid (127 mmol, 1 equiv.) were added. A solution of DCC (26.3 g, 127 mmol, 1 equiv.) in abs. DMF (130 ml) was added over 1 h at 0 °C. Clouding occured and the reaction was continued at room temperature for 16 h. The urea formed was filtered off, the filtrate was concentrated in vacuo, and subsequently a little portion of $CHCl_3$ was added. After storage at −20 °C for 1 h the residual urea was filtered off and the filtrate was dialysed for 48 h in $CHCl_3$.

4.2.1. (Diethylphosphono)acetic acid polyglycerylester 5. Conversion: 91%; yield: 83%; ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.20–5.00 (polyglycerol linear units), 4.40–4.10 (polyglycerol terminal units), 4.10 (m, 4H, 1′-H/1″-H), 3.80–3.20 (polyglycerol), 2.95 (d, J=21.1 Hz, 2H, 2-H), 1.28 (t, J=7.0 Hz, 6H, 2′-H/2″-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=165.5 (1-C), 79.5–62.0 (polyglycerol), 63.0 (1′-C/1″-C), 34.2 (2-C), 16.5 (2′-C/2″-C); ^{31}P NMR (121 MHz, $CDCl_3$): δ=20.5 (P); IR (KBr): ν=3700–3050, 2931, 2243, 1740, 1670, 1382, 1260, 1115, 1026, 976, 911, 738, 647 cm^{-1}.

4.2.2. Cinnamic acid polyglyceryl ester 8f. Conversion: 38%; yield: quant.; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.76–7.54 (d, 1H, 3-H), 7.54–6.88 (m, 5H, Ar–H), 6.43 (d, $J_{2,3}$=$J_{3,2}$=12.4 Hz, 1H, 2-H), 5.45–4.95 (polyglycerol linear units), 4.80–3.00 (polyglycerol), 0.78 (polyglycerol starter); ^{13}C NMR (75 MHz, $CDCl_3$): δ=166.9 (1-C), 145.5 (3-C), 134.2 (4-C), 131.4 (7-C), 129.9 (5-C/9-C), 127.3 (6-C/8-C), 118.5 (2-C), 82.7–62.0 (polyglycerol); IR (KBr): ν=3400–3150, 3150–2500, 1712, 1637, 1578, 1450, 1311, 1172, 865, 767, 684 cm^{-1}.

4.2.3. *p*-Bromocinnamic acid polyglyceryl ester 8h. Conversion: 53%; yield: 99%; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.63–7.00 (m, 5H, 3-H/Ar–H), 6.38 (d, 1H, 2-H), 5.49–5.02 (polyglycerol linear units), 4.67–2.98 (polyglycerol), 0.86 (polyglycerol starter); ^{13}C NMR (75 MHz, $CDCl_3$): δ=166.1 (1-C), 144.0 (3-C), 133.2 (4-C), 132.3 (6-C/8-C), 129.7 (5-C/9-C), 124.9 (7-C), 118.3 (2-C), 82.0–61.0 (polyglycerol); IR (KBr): ν=3456, 3027, 2921, 1714, 1636, 1587, 1488, 1403, 1310, 1267, 1170, 1072, 1009, 982, 817, 731, 695 cm^{-1}.

4.2.4. *p*-Fluorocinnamic acid polyglyceryl ester 8j. Conversion: 46%; yield: 80%; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.61 (d, $J_{2,3}$=$J_{3,2}$=11.0 Hz, 1H, 3-H), 7.44 (m, 2H, 5-H/9-H), 7.00 (m, 2H, 6-H/8-H), 6.35 (d, $J_{2,3}$=$J_{3,2}$=11.0 Hz, 1H, 2-H), 5.49–5.02 (polyglycerol linear units), 4.98–2.98 (polyglycerol), 2.54 (polyglycerol), 0.78 (polyglycerol starter); ^{13}C NMR (75 MHz, $CDCl_3$): δ=166.3 (1-C), 162.4 (7-C), 144.1 (3-C), 130.2 (5-C/9-C), 117.4 (2-C), 116.2 (6-C/8-C), 81.2–60.3 (polyglycerol); IR (KBr): ν=3446, 2876, 2361, 1716, 1638, 1601, 1510, 1457, 1416, 1316, 1233, 1161, 984, 833, 757, 450 cm^{-1}.

4.2.5. *p*-Methoxycinnamic acid polyglyceryl ester 8k. Conversion: 39%; yield: 71%; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.61 (d, $J_{2,3}$=$J_{3,2}$=13.4 Hz, 1H, 3-H), 7.40 (m, 2H, 5-H/9-H), 6.82 (m, 2H, 6-H/8-H), 6.31 (d, $J_{2,3}$=$J_{3,2}$=13.4 Hz, 1H, 2-H), 5.46–5.00 (polyglycerol linear groups), 5.00–3.02 (polyglycerol), 3.76 (s, 3H, Me–H) 2.75 (polyglycerol), 0.79 (polyglycerol starter); ^{13}C NMR (75 MHz, $CDCl_3$): δ=167.3 (1-C), 161.5 (7-C), 145.0 (3-C), 129.9 (5-C/9-C), 127.1 (4-C), 115.2 (2-C), 114.4 (6-C/8-C), 80.9–60.4 (polyglycerol), 55.4 (Me–C); IR (KBr): ν=3458, 2912, 1709, 1634, 1604, 1575, 1513, 1463, 1423, 1289, 1254, 1171, 1030, 984, 829, 756, 480 cm^{-1}.

4.2.6. 3-(Thien-2-yl)acrylic acid polyglyceryl ester 8l. Conversion: 46%; yield: 79%; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.76 (d, $J_{2,3}$=$J_{3,2}$=14.9 Hz, 1H, 3-H), 7.33 (m, 1H, 7-H), 7.21 (m, 1H, 6-H), 6.99 (m, 1H, 5-H), 6.21 (d, $J_{2,3}$=$J_{3,2}$=14.9 Hz, 1H, 2-H), 5.50–5.01 (polyglycerol linear units), 5.00–2.98 (polyglycerol), 2.53 (polyglycerol), 0.79 (polyglycerol starter); ^{13}C NMR (75 MHz, $CDCl_3$): δ=166.8 (1-C), 139.5 (4-C), 137.8 (3-C), 131.4 (5-C), 128.8 (7-C), 128.2 (6-C), 116.5 (2-C), 81.5–59.9 (polyglycerol); IR (KBr): ν=3442, 2921, 2360, 2342, 1707, 1625, 1454, 1426, 1363, 1305, 1279, 1231, 1204, 1165, 1045, 971, 857, 833, 755, 709 cm^{-1}.

4.3. General procedure for the Horner–Wadsworth–Emmons reaction with polyglycerol supported (diethylphosphono)acetic acid 5 and a carbonyl compound 6

This reaction was performed under an inert gas atmosphere and exclusion of water. In a Schlenk-tube (diethylphosphono)acetic acid polyglycerylester **5** (1.0 g, 4 mmol (diethylphosphono)acetate groups, 1 equiv.) was dissolved in abs. THF (15 ml) upon ultrasonification. The respective carbonyl compound **6** (4.4 mmol, 1.1 equiv.) was added either pure or as a THF solution. While stirring, LDA (4.4 mmol, 1.1 equiv. of a 2 M solution in THF/heptane/ethylbenzene) was added dropwise to the reaction mixture over a period of 2 h. Upon addition of the base, reversible temporary clouding was observed. After additional stirring at room temperature for 12 h, 0.1 M aq. HCl was added. The reaction mixture was concentrated in vacuo and dialysed in $CHCl_3$ for 48 h.

4.3.1. Cyclohexylideneacetic acid polyglyceryl ester 8a. Conversion: 90%; yield: quant.; ^{1}H NMR (300 MHz, $CDCl_3$, sample contains residual polymer bound substrate at δ=4.09, 3.08, 1.37–1.15): δ=5.59 (1H, 2-H), 5.24–4.89 (polyglycerol linear units), 4.39–3.50 (polyglycerol), 2.79 (2H, 4-H), 2.17 (2H, 8-H), 1.58 (6H, 5-H/6-H/7-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=166.5 (1-C), 164.7 (3-C), 112.9 (2-C), 80.4–61.4 (polyglycerol), 38.3 (4-C), 30.2 (8-C), 28.9 (5-C), 28.1 (7-C), 26.5 (1-C); ^{31}P NMR (121 MHz, $CDCl_3$): no phosphorus signals; IR (KBr): ν=3408, 2931, 2857, 1715, 1647, 1449, 1386, 1309, 1271, 1238, 1206, 1155, 1127, 1105, 1031, 851, 800, 754 cm^{-1}.

4.3.2. (4-*tert* Butylcyclohexylidene)acetic acid polyglyceryl ester 8b. Conversion: 90%; yield: 86%; ^{1}H NMR (300 MHz, $CDCl_3$, sample contains residual polymer bound substrate at δ=1.40–1.05): δ=5.59 (1H, 2-H), 4.23–3.33 (polyglycerol), 2.78 (2H, 4-H), 2.16 (2H, 8-H), 1.89 (4H, 5-H/7-H), 1.80 (1H, 6-H), 0.84 (9H, Me); ^{13}C NMR (75 MHz, $CDCl_3$, sample contains residual polymer bound substrate at δ=16.5): δ=166.4 (1-C), 164.4 (3-C), 112.4 (2-C), 79.7–61.0 (polyglycerol), 47.9 (1-C), 38.0 (4-C), 32.6 ($C_{quart.}$), 29.7 (8-C), 29.3 (5-C), 28.5 (7-C), 27.7 (Me); IR (KBr): ν=3398, 2947, 2361, 1716, 1651, 1447, 1394, 1366, 1247, 1184, 1139, 863, 756, 666, 478 cm^{-1}.

4.3.3. (4-Chlorocyclohexylidene)acetic acid polyglyceryl ester 8c. Conversion: 65%; yield: 82%; ^{1}H NMR (300 MHz, $CDCl_3$, sample contains residual polymer bound substrate at δ=4.15, 3.24–2.94, 1.48–1.07): δ=5.65 (1H, 2-H), 5.29–4.85 (polyglycerol linear units), 4.44–3.29 (polyglycerol), 4.27 (1H, 6-H), 2.68 (broad d, 2H, 4-H), 2.28–1.77 (m, 6H, 5-H/7-H/8-H); ^{13}C NMR (75 MHz, $CDCl_3$, sample contains residual polymer bound substrate at δ=16.5): δ=165.5 (1-C), 160.6 (3-C), 114.1 (2-C), 80.5–60.4 (polyglycerol), 58.3 (C-6), 36.9 (4-C), 36.2 (5-C), 34.2 (7-C), 26.1 (8-C); IR (KBr): ν=3365, 2947, 1716, 1651, 1445, 1389, 1263, 1127, 963, 862, 757, 481 cm^{-1}.

4.3.4. (4-Bromocyclohexylidene)acetic acid polyglyceryl ester 8d. Conversion: 55%; yield: quant.; ^{1}H NMR (300 MHz, $CDCl_3$, sample contains residual polymer bound substrate at δ=4.12, 3.11–2.86, 1.30): δ=5.63 (1H, 2-H), 5.27–5.07 (polyglycerol linear units), 4.48–3.11 (polyglycerol), 4.40 (1H, 6-H), 3.05–2.68 (m, 2H, 4-H), 2.67 (broad d, 2H, 4-H), 2.31–1.89 (m, 6H, 5-H/7-H/8-H); ^{13}C NMR (75 MHz, $CDCl_3$, sample contains residual polymer bound substrate at δ=62.99, 16.45): δ=165.7 (1-C), 160.3 (3-C), 114.3 (2-C), 80.9–61.0 (polyglycerol), 51.2 (C-6), 37.7 (4-C), 37.0 (5-C), 35.2 (7-C), 27.2 (8-C); IR (KBr): ν=3387, 2931, 1716, 1652, 1445, 1391, 1260, 1051, 859, 755, 477 cm^{-1}.

4.3.5. (4-Hydroxycyclohexylidene)acetic acid polyglyceryl ester 8e. Conversion: 55%; yield: 43%; ^{1}H NMR (300 MHz, $CDCl_3$, sample contains residual polymer bound substrate at δ=4.12, 2.94, 1.40–1.07 and an impurity at δ=7.37–7.09): δ=5.70 (1H, 2-H), 5.35. −4.94

(polyglycerol), 4.46–3.23 (polyglycerol), 3.91 (1H, 6-H), 2.66–1.49 (m, 8H, 4-H/5-H/7-H/8-H); ^{13}C NMR (75 MHz, $CDCl_3$, sample contains residual polymer bound substrate at δ=62.8, 16.5): δ=166.2 (1-C), 165.9 (3-C), 115.7 (2-C), 80.4–65.4 (polyglycerol), 65.1 (6-C), 37.2–25.4 (4-C/5-C/7-C/8-C); IR (KBr): ν=3360, 2931, 2360, 1715, 1646, 1392, 1231, 1052, 959, 754, 458 cm^{-1}.

4.3.6. Cinnamic acid polyglyceryl ester 8f. Conversion: 65%; yield: quant.; ^{1}H NMR (300 MHz, $CDCl_3$, sample contains residual polymer bound substrate at δ=4.10, 3.09–2.85, 1.25): δ=7.64 (1H, 3-H), 7.57–7.11 (5H, Ar–H), 6.40 (1H, 2-H), 5.44–5.03 (polyglycerol linear units), 4.60–3.14 (polyglycerol); ^{13}C NMR (75 MHz, $CDCl_3$, sample contains residual polymer bound substrate at δ=62.9, 16.4): δ=166.3 (1-C), 145.5 (3-C), 134.4 (4-C), 130.5 (7-C), 129.0 (5-C/9-C), 128.3 (6-C/8-C), 117.7 (2-C), 79.9–62.7 (polyglycerol); IR (KBr): ν=2927, 2361, 1716, 1636, 1450, 1270, 1168, 768, 458 cm^{-1}.

4.3.7. *p*-Chlorocinnamic acid polyglyceryl ester 8g. Conversion: 70%; yield: quant.; ^{1}H NMR (300 MHz, $CDCl_3$, sample contains residual polymer bound substrate at δ=4.11, 3.01, 2.93, 1.28): δ=7.57 (1H, 3-H), 7.41 (2H, 5-H/9-H), 7.30 (2H, 6-H/8-H), 6.91 (1H, 2-H), 5.45–4.96 (polyglycerol linear units), 4.57–3.23 (polyglycerol); ^{13}C NMR (75 MHz, $CDCl_3$, sample contains residual polymer bound substrate at δ=62.9, 35.1, 33.5, 16.5): δ=165.4 (1-C), 144.1 (3-C), 136.4 (4-C), 132.8 (7-C), 129.3 (5-C/6-C/8-C/9-C), 80.2–62.9 (polyglycerol); IR (KBr): ν=3397, 2929, 2361, 1717, 1636, 1593, 1492, 1407, 1270, 1052, 979, 823, 755, 480 cm^{-1}.

4.3.8. *p*-Bromocinnamic acid polyglyceryl ester 8h. Conversion: 80%; yield: 94%; ^{1}H NMR (300 MHz, $CDCl_3$, sample contains residual polymer bound substrate at δ=4.10, 3.01, 2.93, 1.27): δ=7.54 (1H, 3-H), 7.43 (2H, 5-H/9-H), 7.31 (2H, 6-H/8-H), 6.37 (1H, 2-H), 5.44–4.94 (polyglycerol linear units), 4.62–3.19 (polyglycerol); ^{13}C NMR (75 MHz, $CDCl_3$, sample contains residual polymer bound substrate at δ=62.7, 35.1, 33.3, 16.5): δ=166.0 (1-C), 144.2 (3-C), 133.2 (4-C), 132.2 (6-C/8-C), 129.6 (5-C/9-C), 124.8 (7-C), 118.3 (2-C), 80.4–60.9 (polyglycerol); IR (KBr): ν=2929, 2361, 1716, 1636, 1587, 1489, 1403, 1072, 819, 756, 479 cm^{-1}.

4.3.9. *p*-Hydroxycinnamic acid polyglyceryl ester 8i. No product formation observed, even though, 2 equiv. of LDA were used.

4.4. General procedure for the Michael-addition of nitromethane to polyglycerol supported α,β-unsaturated carboxylic acids 8

The polyglycerol supported α,β-unsaturated carboxylic acid **8** (14 mmol, 1 equiv.) was dissolved in THF (50 ml). Upon addition of nitromethane (3 ml, 55 mmol, 4 equiv.) and TBAF·3H_2O (8.71 g, 27.6 mmol, 2 equiv.) the mixture turned reddish brown. It was stirred at 40 °C for 24 h. Reaction control was performed via IR-spectroscopy (disappearance of an alkene band at ≈1650 cm^{-1} upon simultaneous appearance of a nitro band at ≈1550 cm^{-1}). When the reaction was completed, Lewatit® K1131 (13.8 g, 55 mmol, 4 equiv.) and Ionenaustauscher III (Merck) (13.8 g, 55 mmol, 4 equiv.) were added and the mixture was shaken for 2 h on a shaker. The ion exchange resins were filtered off, the filtrate was concentrated in vacuo and dialysed in $CHCl_3$ (although sometimes the product is not completely soluble in this solvent) for 24 h.

4.4.1. (1-Nitromethylcyclohex-1-yl)ethanoic acid polyglyceryl ester 9a. Conversion: 78%; yield: 55%; ^{1}H NMR (300 MHz, $CDCl_3$, sample contains residual substrate at δ=5.64–5.47, 2.93, 2.78, 2.17, 1.97): δ=5.25–4.89 (polyglycerol linear units), 4.65 (2H, 9-H), 4.44–3.06 (polyglycerol), 2.53 (2H, 2-H), 1.50 (10H, 4-H/5-H/6-H/7-H/8-H), 1.22 (polyglycerol starter), 0.82 (polyglycerol starter); ^{13}C NMR (75 MHz, $CDCl_3$, sample contains substrate at δ=38.1, 29.7, 28.5): δ=171.1 (1-C), 81.3 (9-C), 80.0–59.7 (polyglycerol), 39.2 (3-C), 37.0 (2-C), 33.7 (4-C/8-C), 25.5 (6-C), 21.2 (5-C/7-C); IR (KBr): ν=3315, 2960, 2874, 1732, 1545, 1461, 1381, 1270–1069, 883, 740, 651 cm^{-1}.

4.4.2. (4-*tert* Butyl-1-nitromethylcyclohex-1-yl)ethanoic acid polyglycerylester 9b. Conversion: 90%; yield: 48%; ^{1}H NMR (300 MHz, $CDCl_3$): δ=4.58 (1H, 9-H^a), 4.54 (1H, 9-H^b), 4.28–2.97 (polyglycerol), 2.63 (1H, 2-H^a), 2.60 (1H, 2-H^b), 1.96–1.07 (9H, 4-H/5-H/6-H/7-H/ 8-H), 0.85 (9H, *t*Bu-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=171.0 (1-C), 84.3 (9-C), 79.6–67.3 (polyglycerol), 47.4 (6-C), 36.6 (3-C), 33.6 (2-C), 31.3 (4-C/8-C), 27.4 (Me–C), 23.7 (5-C/7-C); IR (KBr): ν=3311, 2960, 2873, 1728, 1604, 1546, 1459, 1380, 1227, 1183, 1109, 1061, 882, 740 cm^{-1}.

4.4.3. 4-Nitro-3-phenylbutanoic acid polyglycerylester 9f. Conversion: quant.; yield: 68%; ^{1}H NMR (300 MHz, CD_3OD): δ=7.26 (5H, Ar–H), 5.35–3.02 (polyglycerol), 4.81 (OH), 4.70 (2H, 10-H), 2.79 (2H, 2-H); ^{13}C NMR (75 MHz, CD_3OD): δ=172.5 (1-C), 139.9 (4-C), 130.0 (5-C/9-C), 129.7 (6-C/8-C), 128.8 (7-C), 82.9–60.4 (polyglycerol), 80.5 (10-C), 41.7 (2-C), 38.7 (3-C). IR (KBr): ν=3406, 2961, 2875, 2066, 1840, 1732, 1626, 1550, 1486, 1470, 1381, 1221, 1152, 1067, 883, 740, 703, 573 cm^{-1}.

4.4.4. 3-(*p*-Bromophenyl)-4-nitrobutanoic acid polyglycerylester 9h. Conversion: quant.; yield: 72%; ^{1}H NMR (400 MHz, $(CD_3)_2CO$): δ=7.48 (2H, 5-H/9-H), 7.31 (2H, 6-H/8-H), 5.63–2.39 (polyglycerol), 4.87 (2H, 10-H), 2.84 (2H, 2-H), 1.46–1.22 (polyglycerol starter), 0.86 (polyglycer starter); ^{13}C NMR (100 MHz, $(CD_3)_2CO$): δ=171.3 (1-C), 139.6 (4-C), 132.6–130.8 (2-C/6-C/8-C/9-C), 122.0 (7-C), 79.8 (10-C), 73.7–64.4 (polyglycerol), 40.7 (2-C), 37.7 (3-C); IR (KBr): ν=3273, 2918, 1734, 1552, 1489, 1377, 1251, 1109, 1075, 1010, 827, 668, 531 cm^{-1}.

4.4.5. 3-(*p*-Fluorophenyl)-4-nitrobutanoic acid polyglycerylester 9j. Conversion: quant.; yield: 24%; ^{1}H NMR (300 MHz, CD_3OD): δ=7.20 (2H, 5-H/9-H), 6.94 (2H, 6-H/8-H), 4.83 (OH/10-H), 4.68–2.19 (polyglycerol), 2.47 (2H, 2-H), 1.46–1.22 (polygylcerol starter), 0.85 (polyglycerol starter); ^{13}C NMR (75 MHz, CD_3OD): δ=178.2 (1-C), 163.4 (7-C), 135.2 (4-C), 130.9 (5-C/9-C), 116.5 (6-C/8-C), 79.6 (10-C) 75.8–58.4 (polyglycerol), 41.9 (2-C), 35.5 (3-C); IR (KBr): ν=3853, 3423, 2963, 2876, 1730, 1636, 1551, 1511, 1486, 1467, 1381, 1224, 1161, 1105, 1067, 883, 840, 739, 650 cm^{-1}.

4.4.6. 3-(*p*-Methoxyphenyl)-4-nitrobutanoic acid polyglycerylester 9k. Conversion: quant.; yield: 60%; ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ=7.22 (2H, 5-H/9-H), 6.84 (2H, 6-H/8-H), 5.46–2.99 (polyglycerol/10-H), 3.70 (Me–H), 2.73 (2H/2-H), 1.57 (polyglycerol starter), 1.41–1.13 (polyglycerol starter), 0.79 (polyglycerol starter); ^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ=170.6 (1-C), 158.5 (7-C) 130.9 (4-C), 128.7 (5-C/9-C), 113.9 (6-C/8-C), 78.7 (10-C), 74.4–59.9 (polyglycerol), 55.0 (Me–C), 37.4 (3-C); IR (KBr): ν=3628, 3384, 2960, 2874, 2048, 1836, 1586, 1547, 1514, 1465, 1381, 1226, 1180, 1111, 1066, 1029, 884, 832, 806, 740 cm^{-1}.

4.4.7. 4-Nitro-3-thien-2′-ylbutanoic acid polyglycerylester 9l. Conversion: quant.; yield: 64%; ^{1}H NMR (300 MHz, $(CD_3)_2SO$, sample contains an impurity at δ=1.35): δ=7.38 (1H, 5-H), 6.98 (2H, 6-H/7-H), 5.53–3.03 (polyglycerol/8-H), 2.83 (2-H), 1.57 (polyglycerol starter), 1.35–1.09 (polyglycerol starter), 0.79 (polyglycerol starter); ^{13}C NMR (75 MHz, $(CD_3)_2SO$, sample contains an impurity at δ=30.4): δ=170.3 (1-C), 141.7 (4-C), 127.0 (7-C), 125.2 (5-C), 79.6 (6-C), 77.9 (8-C), 75.0–59.4 (polyglycerol), 38.0 (3-C), 35.2 (2-C); IR (KBr): ν=3417, 2960, 2874, 2046, 1835, 1596, 1549, 1464, 1382, 1225, 1066, 1030, 883, 739 cm^{-1}.

4.5. General procedure for the cleavage of GABA-lactam analogues 4 from the polyglycerol support: reduction of polyglycerol supported γ-nitro acids 9

The polygylcerol supported γ-nitroacid **9** (0.4 g) was dissolved in ethanol or methanol (30 ml). Zinc powder (1 g) and conc. aq. HCl (1 ml) was added and the mixture was refluxed for 1.5–4 h. Upon longer reaction times byproducts were observed. Reaction control was performed via TLC (TBME/MeOH, 95:5, detection: UV/$KMnO_4$). After complete conversion the reaction mixture was filtered over cellite and the filtrate was concentrated in vacuo and purified via filtration over silica (**4a**,**f**), column chromatography (**4b**) (eluent: TBME/MeOH 95:5) or HPLC (**4j**,**l**) (reversed phase C-18, eluent: MeOH/H_2O, 55:45) (depending on the separation problem).

4.5.1. 2-Aza-spiro[4.5]decan-3-on (GABA-pentin-lactam) 4a. Yield (=overall conversion): 58%; ^{1}H NMR (300 MHz, CD_3OD): δ=7.16 (NH), 3.19 (s, 2H, 1-H), 2.27 (s, 2H, 4-H), 1.73–1.24 (m, 10H, 6-H/7-H/8-H/9-H/10-H); ^{13}C NMR (75 MHz, CD_3OD): δ=181.0 (3-C), 54.7 (1-C), 43.6 (5-C), 40.0 (4-C), 36.7 (6-C/10-C), 25.8 (8-C), 23.1 (7-C/9-C); IR (KBr): ν=3204, 3098, 2928, 2870, 2844, 1699, 1675, 1493, 1452, 1420, 1380, 1320, 792, 691, 537, 501 cm^{-1}; FAB-MS (LR, 70 eV): m/z=154.2 $[M+H]^+$; HRMS: calcd for $C_9H_{16}ON$ $[M+H]^+$ 154.1154, found 154.1248.

4.5.2. 2-Aza-8-*tert* butyl-spiro[4.5]decan-3-on (8-*tert* butyl-GABA-pentin-lactam) 4b. Yield (=overall conversion): 70%; ^{1}H NMR (400 MHz, CD_3OD): δ=3.27 (s, 2H, 1-H), 3.10 and 2.25 (s, 2H, 4-H), 1.81–1.59 and 1.40–0.91 (m, 8H, 6-H/7-H/9-H/10-H), 0.81 (s, 9H, *t*Bu-H); ^{13}C NMR (100 MHz, CD_3OD): δ=180.7 (3-C), 57.0 (1-C), 49.1 (8-C), 41.4 (5-C), 40.3 (4-C), 37.8 (6-C/10-C), 32.9 (*t*Bu-C), 27.7 (Me–C), 24.6 (7-C/9-C); IR (KBr): ν=3589, 3518, 3376, 2940, 2866, 1649, 1606, 1483, 1450, 1415, 1393, 1365, 1331, 1238, 1142, 1040, 947, 725, 677, 628 cm^{-1}; FAB-MS (LR, 70 eV): m/z=210.2 $[M+H]^+$; HRMS: calcd for $C_{13}H_{24}ON$ $[M+H]^+$ 210.1780, found 210.1884.

4.5.3. 4-Phenyl-azolidin-2-one (4-phenyl-GABA-lactam) 4f. Yield (=overall conversion): 36%; ^{1}H NMR (300 MHz, CD_3OD): δ=7.58 (m, 5H, Ar–H), 4.17–3.93 (m, 2H, 4-H/5-H^a), 3.65 (dd, 1H, 5-H^b), 2.95 (dd, 1H, 3-H^a), 2.78 (dd, 2H, 3-H^b); ^{13}C NMR (75 MHz, CD_3OD): δ=180.6 (2-C), 143–4 (6-C), 129.6 (7-C), 127.8 (9-C/11-C), 127.6 (8-C/10-C), 50.9 (5-C), 41.0 (4-C), 39.2 (3-C); IR (KBr): ν=3490, 2923, 2852, 1647, 1486, 1451, 1267, 1034, 759, 700, 668 cm^{-1}; GC–MS(CI, NH_3): m/z (%)=161.1 (28) $[M]^+$, 133.2 (8) $[M-CO]^+$, 104.2 (100) $[Ph-Et]^+$, 91.2 (12) $[Ph-Me]^+$, 78.2 (31) $[Ph]^+$.

4.5.4. 4-(*p*-Fluorophenyl)-azolidin-2-one (4-(*p*-fluorophenyl)-GABA-lactam 4j. Yield (=overall conversion): 40%; ^{1}H NMR (600 MHz, CD_3OD): δ=7.32 (m, 2H, 7-H/11-H), 7.06 (m, 2H, 8-H/10-H), 3.75 (m, 2H, 4-H/5-H^a), 3.36 (dd, 1H, 5-H^b), 2.67 (dd, 1H, 3-H^a), 2.48 (dd, 1H, 3-H^b); ^{13}C NMR (from correlation experiments): 179.0 (2-C), 162.1 (9-C, J_{FC}=229 Hz), 138.8 (6-C), 128.5 (7-C/11-C), 115.3 (8-C/10-C), 49.7 (5-C), 39.8 (4-C), 38.2 (3-C); IR (KBr): ν=3445, 1684, 1514, 1261, 1222, 1014, 835, 668 cm^{-1}; EI-MS (LR, 70 eV): m/z (%)=179 (37) $[M]^+$, 122 (100) $[F-C_6H_4-Et]^+$; HRMS: calcd for $C_{10}H_{10}FNO$ $[M]^+$ 179.1909, found 179.0716.

4.5.5. (4-(Thien-2′-yl)-azolidin-2-one (4-thien-2′-yl-GABA-lactam) 4l. Yield (=overall conversion): 35%; ^{1}H NMR (600 MHz, CD_3OD, sample contains an impurity not removable by HPLC at δ=1.53, 1.32, 1.18, 0.9): δ=7.26 (m, 1H, 7-H), 6.96 (m, 2H, 8-H/9-H), 4.00 (m 1H, 4-H), 3.79 (dd, 1H, 5-H^a), 3.41 (dd, 1H, 5-H^b), 2.76 (dd, 1H, 3-H^a), 2.50 (dd, 1H, 3-H^b); ^{13}C NMR (from correlation experiments): δ=178.4 (2-C), 146.0 (6-C), 126.7 (8-C), 128.8 (9-C), 123.6 (7-C), 50.2 (5-C), 39.2 (3-C), 36.2 (4-C); IR (KBr): ν=3473, 1959, 1678, 1528, 1441, 1384, 1269, 1180, 1077, 830, 698, 652 cm^{-1}; EI-MS (LR, 70 eV): m/z (%)=167 (18) $[M]^+$, 124 (4) $[M-CO]^+$, 110 (33) $[C_4H_3S-Et]^+$; HRMS: calcd for C_8H_9ONS $[M]^+$ 167.0405, found 167.0397.

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Dendrimers and combinatorial chemistry—tools for fluorescent enhancement in protease assays

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Abstract—FRET based systems are some of the best methods available to detect and monitor proteolytic activity. To enhance fluorescent signals and hence assay sensitivity, two different systems were developed using two different dendrimeric constructs. In the first case, a triple branched dendrimer bearing three dansyl groups was used to enhance assay sensitivity and showed a significant enhancement of fluorescence following enzymatic cleavage. In another example, a *tris*-fluorescein probe, that undergoes self-quenching, was utilized in a combinatorial library synthesis to map the substrate specificity of proteases.

1. Introduction

Proteases are involved in numerous essential biological processes, and one of the first steps in understanding any new protease is the determination of its substrate specificity. Many assays for proteases are based on fluorescent probes due to the high sensitivity of this technique and fluorescence resonance energy transfer (FRET) methods in particular have been extensively exploited. In the FRET method, donor and quencher moieties are separated on a peptide chain to give rise to an internally quenched fluorescent peptide. This fluorescence is regained following cleavage of the peptide by a protease and it is this increase in fluorescence signal that forms the basis of the FRET assay. Many donor/quencher couples have been described in the literature with varying solubilities in aqueous or organic solvents and efficiency of energy transfer.[1]

Multiplying the number of dyes on an internally quenched peptide could increase the sensitivity of the assay and reduce detection levels. Although dendrimers[2] bearing fluorophores have been described in the literature,[3] it is only very recently that research groups have shown their possible use as a highly sensitive tool for biological assay amplification.[4] Multi-dyes derivatives however can show two types of behaviour: (a) significant amplification of fluorescence directly related to the number of chromophores, (b) a self-quenching phenomenon[5] for dendrimers displaying small Stoke's shift fluorophores.

Keywords: FRET; Enzymatic cleavage; Substrate specificity; Combinatorial libraries; Self-quenching.
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Combinatorial chemistry, including 'split and mix' synthesis, represents a key method for the identification and characterisation of new proteases. This methodology has allowed rapid progress to be made in the determination of the optimal proteolytic substrate for a protease[6] and provides essential information for the understanding of complex biological pathways and for the design of inhibitors.[7] We herein describe the use of multilabelled systems to amplify the fluorescent signal following enzymatic cleavage and their application to define the substrate specificity of proteases using combinatorial multi-chromophoric libraries.

2. Results and discussion

2.1. Dendrimers and amplification monomers

A multivalency system bearing a variety of chromophores should be able to reduce the detection limits of bioassays such as protease detection, or increase the length of sequencing runs in fluorescence based sequencing. The AB_3 type monomers, firstly developed by Newkome et al.,[8] were recently extended and enhanced in utility by our group[9,10] and these multivalent compounds were used as a basis to amplify signals by coupling chromophores to the terminal functional groups.[5] Two different strategies were chosen to monitor enzymatic cleavage. The first was based on a FRET tri-donor/mono-quencher system **1** and used Dansyl as the donor and Dabsyl as a quencher. The second involved a self-quenched derivative **2**, using a single fluorophore (fluorescein) in which the peptidic part consisted of a combinatorial library of 1000 peptides (10×10×10) and was used to define protease substrate specificity (Fig. 1).

The amplification isocyanates **8** and **9** were synthesised in six steps as shown in Scheme 1.[10] Thus, the Michael addition of 1,1,1-tris(hydroxymethyl)amino-methane onto acrylonitrile, followed by amino protection (Boc), and reduction of the nitrile groups with BH_3·THF gave **3**. This was reacted with either Dde-OH to give the Dde (2-acetyl-dimedone) protected amine **4** or the Dansyl donor group to give **5**. Following removal of the Boc protecting group, the isocyanates **8** and **9** were prepared following the procedure of Knölker (Scheme 1).[11] Monomer **8** was coupled directly to the resin via the isocyanate and the free amino groups of the amplification monomer were liberated with hydrazine. The fully formed tri-fluorophore construct **9** could be coupled directly to the free amine groups of the resin linked peptide or used in solution phase synthesis.

2.2. FRET-amplified substrate synthesis: 3-donors/1-quencher

The Dansyl/Dabsyl couple was described by Hartwig as a 'couple' to monitor Heck chemistry during catalyst screening.[12] These two dyes are easy to handle in solution or solid phase and display efficient energy transfer properties. Jakubke described the peptidic sequence Gly-Pro-Ala-Lys-Leu-Ala-Ile-Gly as a good substrate for trypsin with a cleavage site between lysine and leucine.[13] Peptides **10a** and **10b** were constructed on solid phase following an Fmoc-strategy using a hydroxymethylphenoxyacetic linker attached to aminomethyl PS resin. The tri-labelled isocyanate **9** was grafted onto the peptides *N*-terminus after a final Fmoc deprotection. The first amino acid (Lys(Ddiv)-OH),[14] at the beginning of the peptide sequence, allowed dabsyl derivatisation after Ddiv-deprotection with hydrazine. A

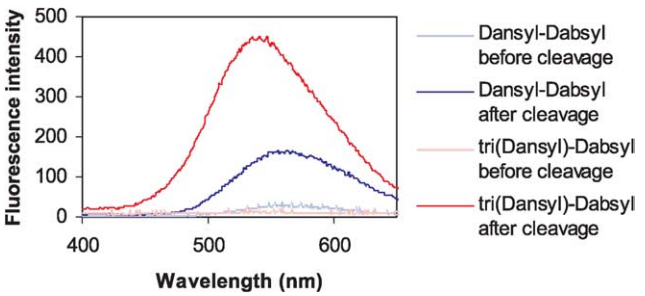


Figure 2. Fluorescence spectra of the peptides **10a** and **10b** (both at 10 μM). Ex=335 nm, Em=545 nm (**10a**) and 560 nm (**10b**). The tri-labelled peptide **10a** (red) showed a much more significant increase in fluorescence than the control peptide **10b** (blue).

control peptide of 1-donor/1-quencher **10b** was also prepared to compare the differences of fluorescence intensity between this and the tri-labelled derivative **10a**. Peptides **10a** and **10b** were precipitated in cold ether, centrifuged and purified by HPLC before analysis (>95%) (Scheme 2).

Enzymatic cleavage was performed on the substrates (excitation at 335 nm and emission at 545 or 560 nm) as shown in Figure 1 and Table 1 demonstrating significant increases in fluorescence in both cases.

Despite the long interchromophore distance, the Dansyl/Dabsyl couple showed good quenching efficiency, which was enhanced for the tribranched-labelled peptide (97%). At equal concentrations (10 μM), the enzymatic cleavage of tri-branched dansyl derivative **10a** (amplification by 28.4) shows a significant enhancement over the control peptide **10b** (amplification by 6.1) and the signal-to-noise ratio

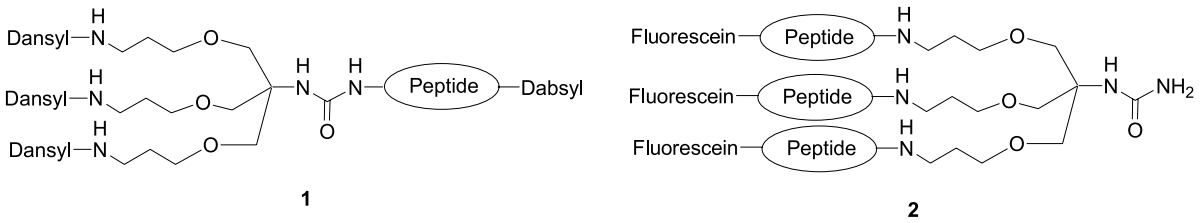
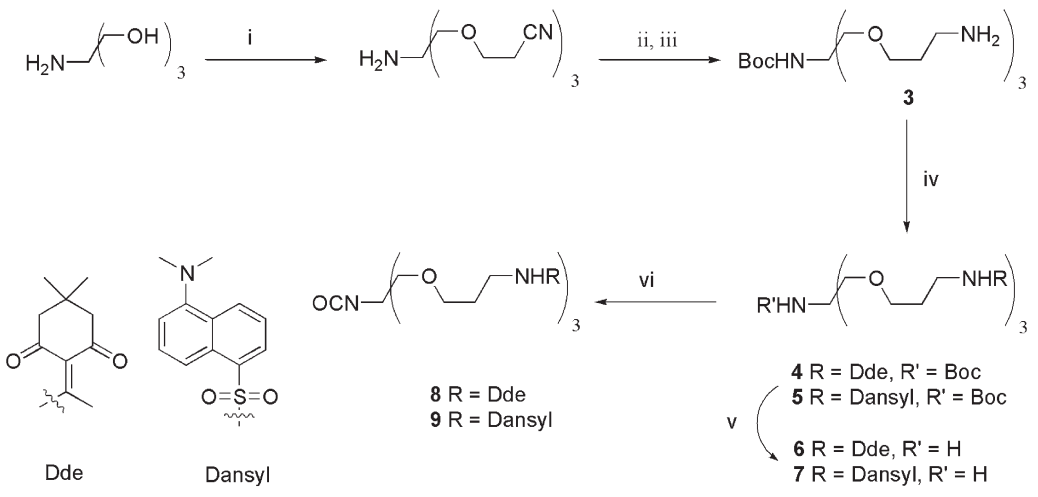
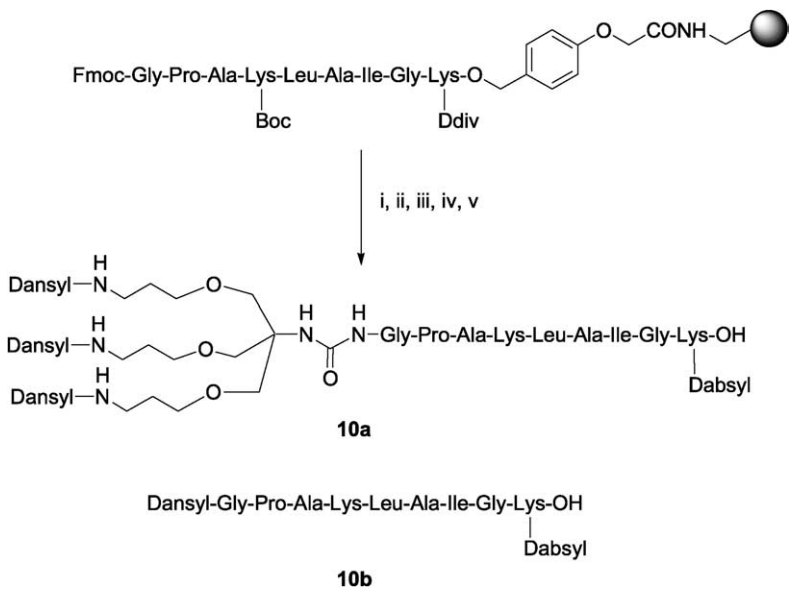


Figure 1. Internally quenched dendritic peptides **1** and **2** which undergo FRET (**1**) and self-quenching (**2**) processes.



Scheme 1. Synthesis of the 1→3 C-branched *tris*-based isocyanate amplification monomers **8** and **9**. (i) acrylonitrile, KOH 40%, dioxane; (ii) Boc_2O, Et_3N; (iii) BH_3·THF, dioxane, 55 °C; (iv) DdeOH, DIPEA for **4** or Dansyl chloride, Et_3N for **5**; (v) 20% TFA in CH_2Cl_2; (vi) Boc_2O, DMAP.



Scheme 2. Synthesis of the FRET-amplified substrate **10a** and the control peptide **10b**. (i) 20% piperidine; (ii) for **10a**: monomer **9**, DIPEA, DMAP. For **10b**: Dansyl-Cl, NEt_3; (iii) 2% hydrazine in DMF; (iv) Dabsyl-Cl, NEt_3; (v) TFA/CH_2Cl_2/TIS 95/3/2.

increased by a factor 4.6 (Fig. 2). Clearly, in this case, the FRET-amplified substrate was not subjected to a self-quenching process, due to its long Stoke's shift.

2.3. FRET-amplified substrate synthesis: self-quenching system

Another approach for assaying protease activity relies on multiple copies of a single type of fluorophore, giving rise internally quenched fluorescent peptides. This method offers a very simple method of 'internal FRET' and was initially described in our group as a method of assaying proteases but can be applied in variety of biological assays. The self-quenching process occurs for small Stoke's shift dyes, when the emission wavelength and the excitation wavelength of the fluorophore are close to each other (4(5)-carboxyfluorescein λ_{ex}=495 nm, λ_{em}=520 nm). In effect, the dendrimer generates a high-local concentration of fluorophore permitting efficient quenching while peptide hydrolysis releases multiple copies of the dyes causing a large increase in emission.

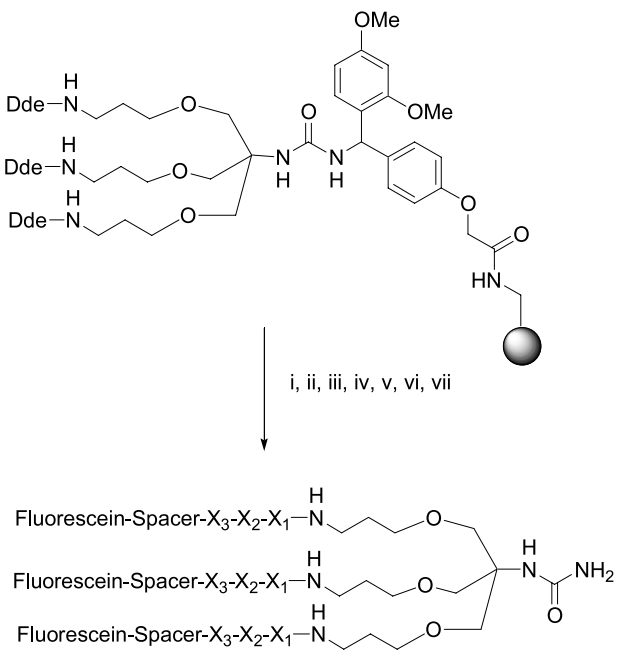
Herein, we show for the first time, that this process of 'internal quenching' can be used in a combinatorial chemistry screening sense, to profile protease specificity. FRET-based libraries of course have been widely exploited to investigate the design of the preferred enzyme substrate,[15,6] but a single fluorophore approach would simplify the whole process, the substrates would be chemically much more accessible, and cleavage could occur anywhere in the peptide chain.

Table 1. Fluorescence intensity of the peptides **10a** and **10b** before and after adding trypsin (arbitrary units)

Peptide	Starting fluorescence	Final fluorescence	Quenching efficiency	Amplification
10b	27	165	84	6.1
10a	16	455	97	28.4

The ability of an enzyme to recognise a substrate selectively in a pool of thousands of potential substrates provides vital information about its mode of action. Split and mix synthesis makes the synthesis rapid and subsequent screening of thousands of peptide targets possible.[16,17]

Here, the determination of the substrate specificity of two model proteases, papain and trypsin is reported by the



Scheme 3. Synthesis of the self-quenched substrate **2** (i) 2% hydrazine in DMF; (ii) (a) Fmoc-amino acid X_1 (10 equiv.), HOBt (10 equiv.), DIC (10 equiv.), (b) 20% piperidine in DMF; (iii) (a) Fmoc-amino acid X_2 (5 equiv.), HOBt (5 equiv.), DIC (5 equiv.), (b) 20% piperidine in DMF; (iv) (a) Fmoc-amino acid X_3 (5 equiv.), HOBt (5 equiv.), DIC (5 equiv.), (b) 20% piperidine in DMF; (v) (a) γ-aminobutyric acid (2 equiv.), HOBt (2 equiv.), DIC (2 equiv.) (b) 20% piperidine in DMF; (vi) 4(5)-carboxyfluorescein (10 equiv.), HOBt (10 equiv.), DIC (10 equiv.); (vii) TFA/TIS/CH_2Cl_2 (95/2/3).

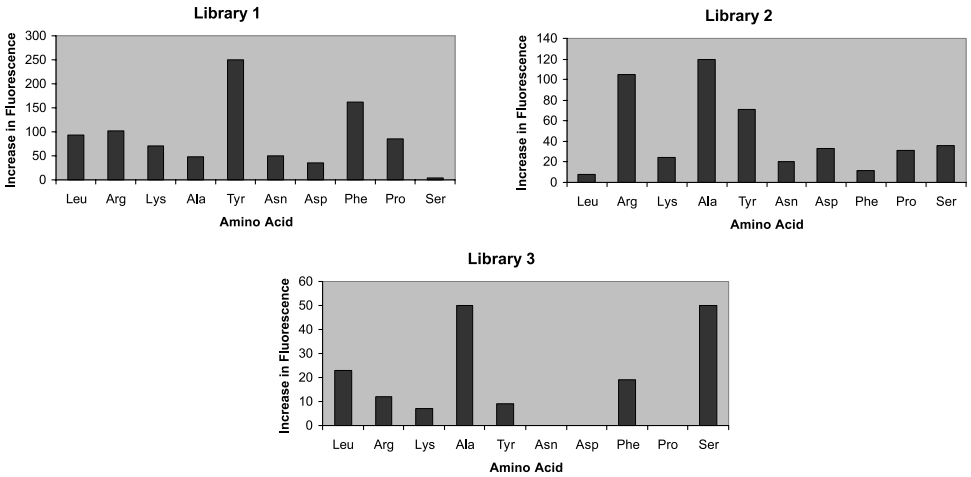


Figure 3. Screening of the 30 self-quenching sub-libraries against papain.

screening of an internally quenched combinatorial peptide library. Three tripeptide sub-libraries were synthesised on the tri-branched resin using the split and mix method (Scheme 3). Monomer **8** was coupled to the Rink linker attached to the aminomethyl PS resin. This resin was deprotected with hydrazine (2% in DMF) and divided into 10 equal sized pools. The X_3 position was fixed in the first library, X_2 in the second library and X_1 position in the third library. To minimize the size of the libraries, ten amino acids with differing properties were chosen (Ala, Arg, Asn, Asp, Leu, Lys, Phe, Pro, Ser and Tyr). Although quite simple, logistically it becomes complex as 100 individual reactions have to be carried out at this split stage. The first amino acid was coupled following standard Fmoc strategy but because of the bulk of the dendrimer backbone, the reactivity of the terminal amine group was affected and some coupling reactions were performed a second time to ensure complete reaction. The second and the third amino acids were attached following the same procedure. The introduction of a spacer was vital; too long results in a decrease of the self-quenching efficiency and too small affects the access of the enzyme to the cleavage site. A 4-carbon spacer, γ-aminobutyric acid, was therefore attached to the backbone (a previous study showed poor hydrolysis kinetics for peptides on dendrimers without spacers). Carboxyfluorescein was finally coupled onto the *N*-termini of all the 30 sub-libraries. The multi labelled peptides were released from the resin with TFA removing, at the same time, side chain protecting groups. The sub-libraries were dissolved in buffer and the concentrations of each of the 30 sub-libraries (each of which contained 100 different tripeptides) were checked by UV spectroscopy (495 nm) to ensure identical concentrations of peptides in each assay.

2.4. Peptide libraries screenings

The 30 sub-libraries were screened against papain, with excitation at 495 nm (fluorescence at 520 nm). Fluorescent enhancement was monitored for 20 min after adding the enzyme (Fig. 3). Library 1 (X_3 fixed) showed selective cleavage for Tyr and Phe. The second library (X_2 fixed) demonstrated a preference for Ala, Arg and Tyr and the third library (X_1 fixed) for small amino acids such as Ser and Ala. These results were consistent with the literature,[18] (with library 1 representing the P_2 position, library 2 the P_1 position and library 3 the P_1' position).

To demonstrate the further potential of the library, the library was screened against another enzyme, trypsin. The results of the screening showed a high preference for basic

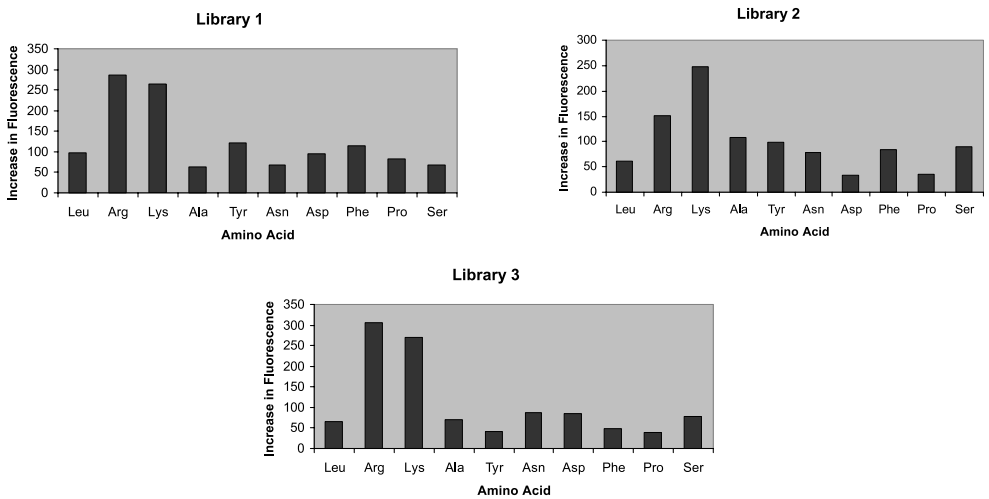


Figure 4. Screening of the 30 self-quenching sub-libraries against trypsin.

amino acid residues (Arg and Lys) as cleavage points in all three sub-libraries (Fig. 4). Trypsin reaches its full hydrolytic efficiency with positively charged side chain residues and presumably, this factor is causing bias with cleavage occurring at all three positions regardless of their location making it difficult to get reasonable data for the specific cleavage site a limitation of the method that has already been observed in other systems.[18]

3. Conclusion

A tri-branched amplification monomer has been developed as a multi-dye carrier to enhance fluorescence signals during protease assays. Peptides bearing a tri-dansyl derivative, internally quenched by a dabsyl group in a peptide, showed a significant increase in fluorescence following hydrolysis. A new 'self-quenched' split and mix peptide library was designed using fluorescein as the 'internally quenched dye', avoiding the need to use two fluorophores as in traditional FRET based protease substrates. Using this 'quenched' split and mix library, the substrate specificity (P_2 to P_1') of papain could be rapidly determined by screening the split and mix sub-libraries.

4. Experimental

4.1. General information

NMR spectra were recorded using Bruker AC 300 or DPX 400 spectrometers operating at 300 or 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts are reported on the δ scale in ppm and are referenced to residual non-deuterated solvent resonances. Electrospray mass spectra were obtained on a VG Platform single quadripole mass spectrometer. MALDI spectra were recorded on a Micromass Tofspec 2E reflection matrix assisted laser desorption ionisation time of flight (MALDI-TOF) mass spectrometer. IR spectra were obtained on a Biorad FTS 135 spectrometer with a Golden Gate accessory with neat compounds as oil or solids. Fluorescent measurements were recorded using a Perkin Elmer Luminescence Spectrometer LS50B. Commercially available reagents were used without further purification. THF was freshly distilled under nitrogen from a solution of sodium and benzophenone. Purifications by column chromatography were carried out on silica gel 60 (230–400 mesh) purchased from Merck. Analytical HPLC was performed on a Hewlett Packard HP1100 Chemstation equipped with a C_{18} ODS analytical column, (4.6×3 mm i.d. 5 μm, flow rate 0.5 ml min^{-1}) eluting with H_2O/MeCN/TFA (90/10/0.1) to H_2O/MeCN/TFA (10/90/0.05) over 3 min, detection by UV at 254 nm. Semi-preparative HPLC was performed on a HP1100 system equipped with a Phenomenex Prodigy C_{18} reverse phase column (250×10.0 mm, flow rate 2.5 ml min^{-1}) eluting with water (0.1% TFA) to MeCN (0.042% TFA) over 20 min. Resin samples were agitated by spinning on a blood-tube rotor. Compounds **3**, **4** and **8** were synthesized according to literature procedures.[10]

4.1.1. [2-{3-[5-Dimethylaminonaphthalene-1-sulfonic acid]propoxy amide}-1,1-bis-{3-[5-dimethylaminonaphthalene-1-sulfonic acid]propoxymethyl amide}-ethyl] carbamic acid *tert*-butyl ester (5). A solution of **3** (400 mg, 1 mmol), dansyl chloride (891 mg, 3.3 mmol, 1.1 equiv.) and triethylamine (335 mg, 3.3 mmol, 1.1 equiv.) in CH_2Cl_2 (20 ml) was stirred for 4 days. The residue was poured into brine (20 ml) and extracted with CH_2Cl_2 (2×10 ml). The combined organic layers were washed with water (20 ml), dried over $MgSO_4$ and the solvent removed *in vacuo*. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 98:2) to give **5** (524 mg, 48%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (s, 9H), 1.58 (quint, J=6.0 Hz, 6H), 2.80 (s, 18H), 2.94 (t, J=6.0 Hz, 6H), 3.22 (s, 6H), 3.38 (t, J=5.5 Hz, 6H), 5.50 (br s, 3H), 7.08 (d, J=7.0 Hz, 3H), 7.38–7.49 (m, 6H), 8.15 (d, J=7.5 Hz, 3H), 8.24 (d, J=8.5 Hz, 3H), 8.44 (d, J=8.5 Hz, 3H); ^{13}C NMR (100 MHz $CDCl_3$) δ 27.5, 28.2, 40.7, 44.6, 52.6, 57.5, 69.0, 69.6, 78.7, 114.4, 118.2, 122.4, 127.5, 128.7, 128.9, 129.1, 129.4, 134.2, 151.1, 154.1; IR υ 3290, 1716, 1666, 1580; MS (ES$^+$): 1092 (M+H)$^+$.

4.1.2. [2-{3-[5-Dimethylaminonaphthalene-1-sulfonic acid]propoxy amide}-1,1-bis-{3-[5-dimethylaminonaphthalene-1-sulfonic acid]propoxymethyl amide}-ethyl] amine (7). The protected amine **5** (524 mg, 0.48 mmol) was treated with 20% TFA in CH_2Cl_2 (10 ml) and stirred for 45 min. The solvent was removed in vacuo and CH_2Cl_2 (50 ml) was added to the crude product which was washed with saturated aqueous $NaHCO_3$ (2×50 ml) and water (50 ml). The organic layer was dried over Na_2SO_4 and the solvent was removed in vacuo to give the amine **7** as a white solid (470 mg, quantitative yield) which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.59 (quint, J=6.0 Hz, 6H), 2.50 (br s, 2H), 2.80 (s, 18H), 2.96 (t, J=6.0 Hz, 6H), 3.22 (s, 6H), 3.40 (t, J=5.5 Hz, 6H), 6.0 (br s, 3H), 7.07 (d, J=7.0 Hz, 3H), 7.42 (dt, J=7.5, 4.0 Hz, 6H), 8.15 (d, J=7.5 Hz, 3H), 8.24 (d, J=8.5 Hz, 3H), 8.44 (d, J=8.5 Hz, 3H); ^{13}C NMR (100 MHz $CDCl_3$) δ 28.0, 40.3, 44.4, 56.4, 68.7, 70.8, 114.2, 118.1, 122.2, 127.2, 128.2, 128.6, 128.9, 129.1, 134.2, 150.8; IR υ 3290, 1662, 1565; MS (ES$^+$): 992 (M+H)$^+$.

4.1.3. [2-{3-[5-Dimethylaminonaphthalene-1-sulfonic acid]propoxy amide}-1,1-bis-{3-[5-dimethylaminonaphthalene-1-sulfonic acid]propoxymethyl amide}-ethyl] isocyanate (9). 7 (470 mg, 0.48 mmol) and DMAP (61 mg, 0.5 mmol) were dissolved in THF (20 ml) and stirred at −10 °C for 5 min under nitrogen To this solution was added dropwise a solution of Boc_2O (152 mg, 0.7 mmol) in THF (2 ml). The reaction mixture was stirred for 90 min and the solvent removed *in vacuo*. Because of its instability, the crude product was rapidly used without further purification for coupling to the peptide. IR υ 2253, 1732, 1609. LC-MS (ES$^-$): 1016 (M−H)$^-$.

4.2. Peptide synthesis

Peptide synthesis was carried out using a hydroxylmethyl-phenoxy-acetic acid linker attached to aminomethyl PS resin (1.11 mmol g^{-1}, 1% DVB, 75–150 μm).

Fmoc-amino acids (3.76 mmol, 2 equiv.) (Fmoc-Lys(Ddiv)-OH (2.15 g), Fmoc-Gly-OH (1.12 g), Fmoc-Ile-OH

(1.33 g), Fmoc-Ala-OH (1.17 g), Fmoc-Leu-OH (1.3 g), Fmoc-Lys(Boc)-OH (1.76 g), Fmoc-Ala-OH (1.17 g), Fmoc-Pro-OH (1.27 g), Fmoc-Gly-OH) (1.12 g) and HOBt (507 mg, 3.76 mmol) were dissolved in CH_2Cl_2 (10 ml) (with enough DMF to get complete dissolution) and stirred at room temperature for 10 min. DIC (0.63 ml, 3.76 mmol) was then added and the resulting solution stirred for a further 10 min. The solution was then added to the resin (approx. 2 g, 1.88 mmol), pre-swollen in CH_2Cl_2, and the reaction mixture agitated for 2 h. The solution was then drained and the resin washed with DMF (×3), MeOH (×3) and CH_2Cl_2 (×3). The coupling reactions were monitored by the qualitative ninhydrin test with the exception of the coupling onto proline which was monitored by a chloranil test. A small amount of resin was cleaved with TFA/CH_2Cl_2/TIS (95/3/2) ratio and analyzed by analytical HLPC (254 nm) and mass spectroscopy to check peptide synthesis.

4.3. Fmoc-deprotection

To the resin (preswollen in CH_2Cl_2) was added 20% piperidine in DMF (15 ml) and the reaction mixture agitated for 20 min. The solution was then drained and the resin was washed with DMF (×3), MeOH (×3) and CH_2Cl_2 (×3).

4.4. Dansyl chromophores coupling

Peptide **10a**. Isocyanate **9** (335 mg, 0.33 mmol), DMAP (2 mg, 0.02 mmol) and DIPEA (64 mg, 0.33 mmol) were dissolved in CH_2Cl_2/DMF (1:1) (4 ml). The resulting solution was added to the resin (500 mg, 0.22 mmol) and the mixture was shaken overnight and monitored by a qualitative ninhydrin test. The solution was then drained and the resin was washed with DMF (×3), MeOH (×3) and CH_2Cl_2 (×3).

Peptide **10b**. Dansyl chloride (119 mg, 0.44 mmol) and triethylamine (44 mg, 0.44 mmol) were added to the pre-swollen resin (500 mg, 0.22 mmol) in CH_2Cl_2 and the reaction mixture was agitated overnight. The solution was then drained and the resin was washed with DMF (×3), MeOH (×3) and CH_2Cl_2 (×3). The coupling reaction was checked by a qualitative ninhydrin test.

4.5. Ddiv deprotection

To the resin (pre-swollen in CH_2Cl_2) (approx. 500 mg) was added 2% hydrazine in DMF (5 ml) and the reaction mixture agitated for 2 h. The solution was then drained and the resin was washed with DMF (×3), MeOH (×3) and CH_2Cl_2 (×3).

4.6. Dabsyl group coupling

Dabsyl chloride (142 mg, 0.44 mmol) and triethylamine (44 mg, 0.44 mmol) were added to the pre-swollen resin (approx. 500 mg, 0.22 mmol) in CH_2Cl_2 and the reaction mixture was agitated overnight. The solution was then drained and the resin was washed with DMF (×3), MeOH (×3) and CH_2Cl_2 (×3). The coupling reaction was checked by a qualitative ninhydrin test.

4.7. TFA cleavage

The resin (approx. 500 mg) was swollen in CH_2Cl_2 and treated with TFA/CH_2Cl_2/TIS (95:3:2) (12 ml) for 45 min. The solution was drained and the resin was washed with the cleavage cocktail (2×5 ml) and the mixture solution was removed in vacuo.

4.8. Purification of peptides 10a and 10b

The crude cleaved peptides **10a** and **10b** were dissolved in the minimum amount of cleavage cocktail and added dropwise to ice-cooled Et_2O. The mixture was centrifuged, the solvent was removed by decantation and the precipitate was washed with Et_2O (×3), before drying in vacuo. The precipitate was purified by RP-HPLC and lyophilized to afford the peptides as red solids.

Peptide **10a**: Yield: 32%. HPLC (254 nm): 3.98 min. MALDI-TOF-MS: 2159 $(M+H)^+$.

Peptide **10b**: Yield: 42%. HPLC (254 nm): 3.52 min. LC-MS: 1374 $(M+H)^+$.

4.9. Library synthesis

The libraries were carried out using a Rink linker attached to aminomethyl PS resin (1.11 mmol g^{-1}, 1% DVB, 75–150 μm). Isocyanate **8** was synthesized according to literature procedures[10] and coupled to the resin (5.0 g, 4.15 mmol). The Dde protecting group was cleaved using 5% hydrazine in DMF (20 ml) for 2 h, the resin was drained and washed using DMF (×3), MeOH (×3), CH_2Cl_2 (×3) and Et_2O (×3).

4.10. Library 1

The resin was divided into 10 portions (each 30 mg, 60 μmol, 2.0 mmol g^{-1} loading). HOBt (81 mg, 600 μmol) was dissolved in CH_2Cl_2/DMF (200 μL: 200 μL) along with one of each of the following amino acids (600 μmol): Fmoc-Pro-OH (202 mg), Fmoc-Ser(^tBu)-OH (230 mg), Fmoc-Asn(Trt)-OH (358 mg), Fmoc-Asp-(O^tBu)-OH (246 mg), Fmoc-Tyr(^tBu)-OH (275 mg), Fmoc-Arg(Pbf)-OH (388 mg), Fmoc-Lys (Boc)-OH (281 mg), Fmoc-Phe-OH (232 mg), Fmoc-Ala-OH (186 mg) and Fmoc-Leu-OH (212 mg). DIC (100 μL, 600 μmol) was added to each mixture. The resulting solution stirred for a further 10 min and added to the pre-swollen resins. The 10 resin samples were shaken overnight. The reaction mixture was drained off and washed using DMF (×3), MeOH (×3), CH_2Cl_2 (×3) and Et_2O (×3). The ninhydrin test was still positive for some of pools and the coupling was repeated under the same conditions as above until completion of reaction. The resin was then combined, mixed and Fmoc cleavage was performed as previously described. The resin was split into 10 portions and the same procedure was employed in order to couple each one of the 10 amino acids onto the second and third positions. Following the coupling of the third amino acid, the pools of resin were labelled according to their third position and the Fmoc group was cleaved using 20% piperidine in DMF.

4.11. Library 2

The resin was divided into 10 portions (each 100 mg, 200 μmol, 2.0 mmol g^{-1} loading). HOBt (270 mg, 2 mmol) was dissolved in CH_2Cl_2/DMF (600 μL: 600 μL) along with one of each of the following amino acids (2 mmol): Fmoc-Pro-OH (674 mg), Fmoc-Ser(*t*Bu)-OH (766 mg), Fmoc-Asn(Trt)-OH (1.20 g), Fmoc-Asp-O*t*Bu (823 mg), Fmoc-Tyr(*t*Bu)-OH (918 mg), Fmoc-Arg(Pbf)-OH (1.30 g), Fmoc-Lys(Boc)-OH (936 mg), Fmoc-Phe-OH (774 mg), Fmoc-Ala-OH (622 mg) and Fmoc-Leu-OH (706 mg). DIC (315 μL, 2 mmol) was added to each mixture. The resulting solutions were stirred for a further 10 min and added to the pre-swollen resin. The pools of resin were shaken overnight. The solution was drained off and the resin washed using DMF (×3), MeOH (×3), CH_2Cl_2 (×3) and Et_2O (×3). The ninhydrin test was still positive for some of pools and the coupling was repeated as above until completion of the reaction. The resin was then combined and mixed and Fmoc cleavage was performed as previously described. The resin was split into 10 portions. The same procedure as above was employed in order to couple each of the amino acid (1 mmol) to each of the portions of resin. The solutions were drained off, the resin washed using DMF (×3), MeOH (×3), CH_2Cl_2 (×3) and Et_2O (×3) and dried and 10 Fmoc cleavages performed. Each pool of resin was labelled according to the second position amino acid and each pool split into 10 further pools (100 in total). The third amino acid was coupled employing the same procedure (100 μmol) before recombining back into 10 pools. The Fmoc group was cleaved using 20% piperidine in DMF.

4.12. Library 3

The resin was divided into 10 portions (each 100 mg, 200 μmol, 2.0 mmol g^{-1} loading). HOBt (270 mg, 2 mmol) was dissolved in CH_2Cl_2/DMF (600 μL: 600 μL) along with each of the following amino acids (2 mmol): Fmoc-Pro-OH (674 mg), Fmoc-Ser(*t*Bu)-OH (766 mg), Fmoc-Asn(trt)-OH (1.20 g), Fmoc-Asp-O*t*Bu (823 mg), Fmoc-Tyr(*t*Bu)-OH (918 mg), Fmoc-Arg(Pbf)-OH (1.30 g), Fmoc-Lys(Boc)-OH (936 mg), Fmoc-Phe-OH (774 mg), Fmoc-Ala-OH (622 mg) and Fmoc-Leu-OH (706 mg). DIC (315 μL, 2 mmol) was added to each mixture. The resulting solution stirred for a further 10 min and added to the pre-swollen resin. The pools of resin were shaken overnight. The solutions were drained off and the resin washed using DMF (×3), MeOH (×3), CH_2Cl_2 (×3) and Et_2O (×3). The ninhydrin test was still positive for some of pools and the coupling was repeated as above until completion of the reaction. The pools of resin were labelled according to the first position amino acids and each pool split into 10 sub-pools. The second amino acid was coupled following the same procedure as above. The 10 sub-pools of resin were mixed in accordance with their labels, Fmoc deprotected and each pool was again split into 10 sub-pools. The third amino acid was coupled employing the same procedures as above. The sub-pools were then recombined to give the 10 pools. The Fmoc group was cleaved using 20% piperidine in DMF.

4.13. Spacer coupling

A stock solution of HOBt (2 equiv.) and Fmoc-γ-amino-butyric acid spacer (2 equiv.) in CH_2Cl_2/DMF along DIC (2 equiv.) was prepared. To each pool, a tenth of this stock solution was added and the mixture was shaken overnight. The solutions were drained off, the resin washed using DMF (×3), MeOH (×3), CH_2Cl_2 (×3) and Et_2O (×3). Qualitative ninhydrin tests were negative for all 10 pools. An Fmoc cleavage was performed in each pool.

4.14. 4(5)-Carboxyfluorescein coupling

A stock solution of HOBt (10 equiv.) and 4(5)-carboxy-fluorescein (10 equiv.) in CH_2Cl_2/DMF along DIC (10 equiv.) was prepared. To each pool, a tenth of this stock solution was added and the mixture was shaken for 24 h. The ninhydrin test was still positive for some of pools and the coupling was repeated.

4.15. General procedure for library cleavage

The resins (15 mg) were swollen in CH_2Cl_2 (1 ml) and treated with TFA/CH_2Cl_2/TIS (95:3:2) (0.5 ml) mixture for 45 min. The solutions were drained and the resins were washed with the cleavage cocktail (2×0.5 ml). The residue was evaporated to dryness and DMSO (1 mL) was added. These stock solutions were sonicated for 30 min to facilitate peptide solubilization. All of the pools were diluted by a factor of 300 in HEPES (50 mM, pH 8) or potassium phosphate (50 mM, pH 6.8) buffer depending on the enzyme under investigation and the final concentrations of the peptide were checked by absorbance intensities at 495 nm (the absorbance wavelength of the fluorophore) and adjusted as necessary.

4.16. Enzymatic assays

4.16.1. Trypsin assays. Enzyme activity was monitored at 25 °C in 50 mM HEPES at pH 8.0, 10 mM $CaCl_2$, 100 mM NaCl, DMSO (15% for peptides **10a** and **10b** and less than 1% for the peptide libraries). Reactions were initiated by the addition of trypsin and monitored fluorimetrically for 20 min with excitation at 335 nm (**10a** and **10b**) or 495 nm (libraries) and emission at 545 nm (**10a**), 560 nm (**10b**) or 520 nm (libraries).

4.16.2. Papain assays. Enzyme activity was monitored at 25 °C in potassium phosphate buffer at pH 6.8, cysteine (2 mM), EDTA (1 mM) and the pool of peptides. Reactions were initiated by the addition of papain and monitored fluorimetrically for 20 min with excitation at 495 nm and emission at 520 nm.

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Preparation and evaluation of bipyridyl-tagged reagents and scavengers

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Abstract—The synthesis of three bipyridyl-tagged reagents and one scavenger is described. Of the three reagents, the carbodiimide derivative proved to be effective as a coupling reagent for amide formation and the removal of the coupling side product from the reaction mixture by complexation onto a Cu-derivatised resin has been successfully demonstrated. This purification process was thoroughly optimised using a DOE approach and the procedure subsequently applied to the use of a bipyridyl-tagged amine as an isocyanate scavenger. Preliminary results clearly demonstrate the potential of using chelation tags such as bipyridine units as a means for removing solution phase reagents and scavengers from reaction mixtures providing an attractive alternative to their resin-bound and fluorous-tagged counter-parts.

1. Introduction

In the past few years, technologies and strategies have been developed to produce large numbers of new compounds in a fast, clean and efficient way. Solid-phase organic synthesis is a widely used methodology but unfortunately, there are limitations to this approach, such as long reaction times and difficulties with monitoring the progress of reactions. New strategies have recently been developed in order to combine the benefits of solution-phase chemistry and the key advantages of solid-phase chemistry, using polymer-supported[1] and fluorous-tagged[2] reagents and scavenging agents. Each of these methods still has its disadvantages, the former being limited by slow reaction times and the requirement for excess reagent to be used in many cases, and the latter being expensive and requiring either solvent extraction or chromatography on fluorous silica as a means of purification. An alternative approach has recently been described by Ley and co-workers[3] who found that 4,4′-bis(hydroxymethyl)-2,2′-bipyridine was a suitable tag for substrates when employing a 'catch and release' synthesis strategy, followed by extraction of the bipyridyl tag onto a copper(II) chelated ion exchange resin and simple filtration to yield the desired products (Fig. 1). The benefits of this approach are that the reactions can be carried out in solution and purification achieved using cheap and readily available materials.

Encouraged by the report from Ley et al. we decided to investigate the possibility of using bipyridyl-tagged reagents and scavengers in synthesis. For the sake of simplicity, we opted to use a monofunctionalised bipyridine tag. This paper describes the synthesis of bipyridyl bound coupling reagents such as carbodiimide, HOBt and tetrafluorophenol analogues, and a bipyridyl bound scavenger. The objectives were the preparation of bipyridyl bound reagents and validation of their use in synthesis followed by purification using a Cu-derivatised resin.

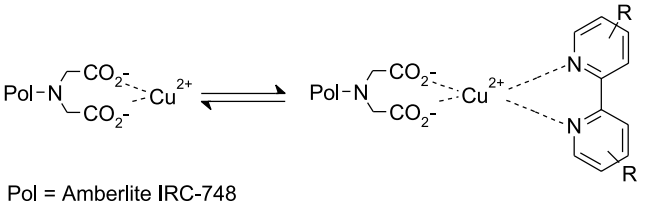

Figure 1. Chelation of bipyridyl unit using Cu-derivatised resin.

2. Results and discussion

Bipyridyl bound carbodiimide **1** and tetrafluorophenol **2** were the initial targets (Fig. 2). It was decided that the bipyridyl unit and the coupling reagent may be bound through an amide linkage, and that a spacer of two or three carbons would be ideal. The proposed synthetic routes involved bipyridyl bound amine **3** as a common intermediate for the synthesis of tetrafluorophenol **4**, hydroxybenzotriazole **5** and carbodiimide **6** (Fig. 3).

Keywords: Bipyridine; Reagent; Scavenger; Chelation-tagged.
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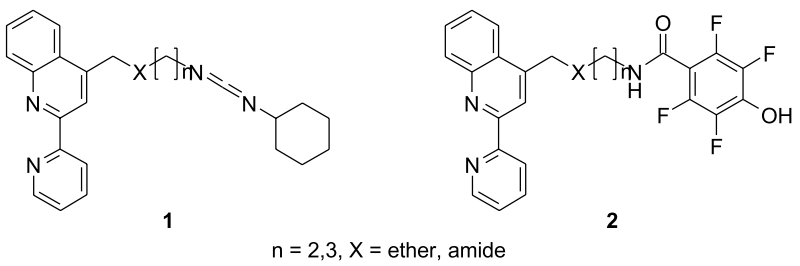


Figure 2.

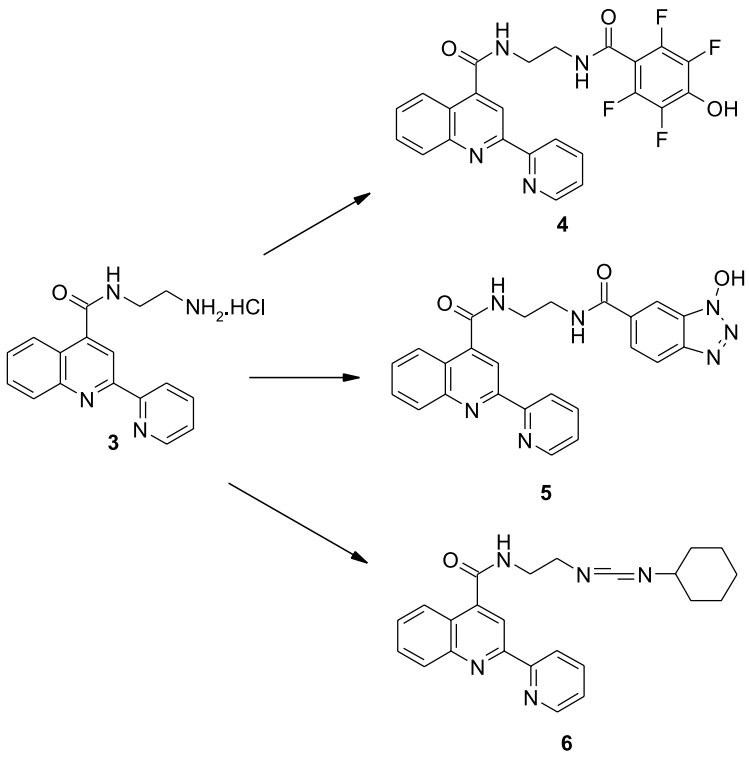
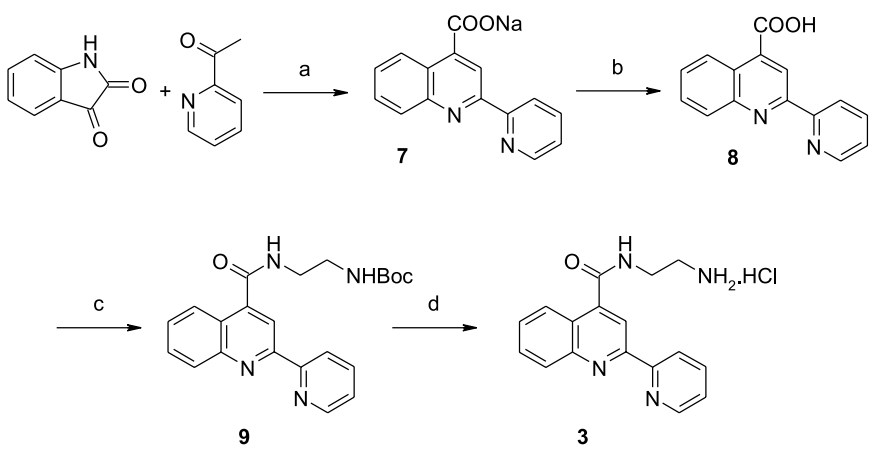
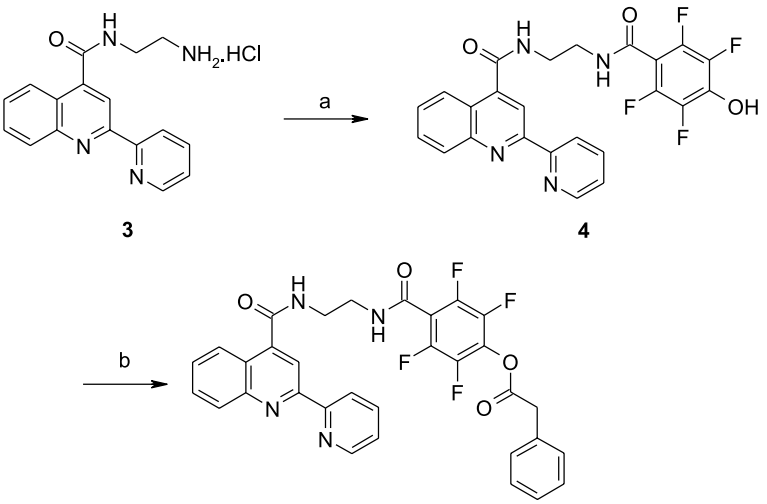


Figure 3.

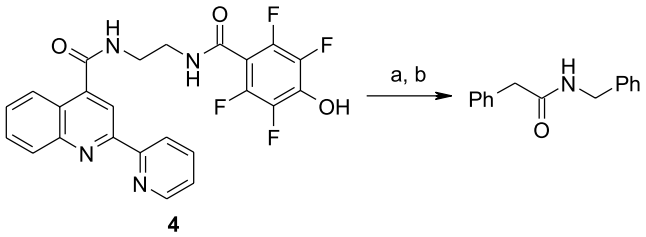
The bipyridyl bound amine **3** was synthesised from the 2-(2-pyridinyl)-4-carboxyquinoline **8**, easily obtained in two steps from commercial compounds according to the procedures described in a patent.[4] The bipyridyl bound amine **3** was then obtained through the coupling between the acid **8** and *N*-Boc-ethylenediamine (Scheme 1). The coupling step was quantitative and the HCl salt of the amine was easily obtained as a white solid upon treatment of compound **9** with HCl/Et_2O.



Scheme 1. (a) 33% NaOH, 62%; (b) 10% HCl, 89%; (c) *N*-Boc-ethylenediamine, EDC, HOBt, DMF, rt, 100%; (d) HCl 2 M/Et_2O, DCM, rt 100%.



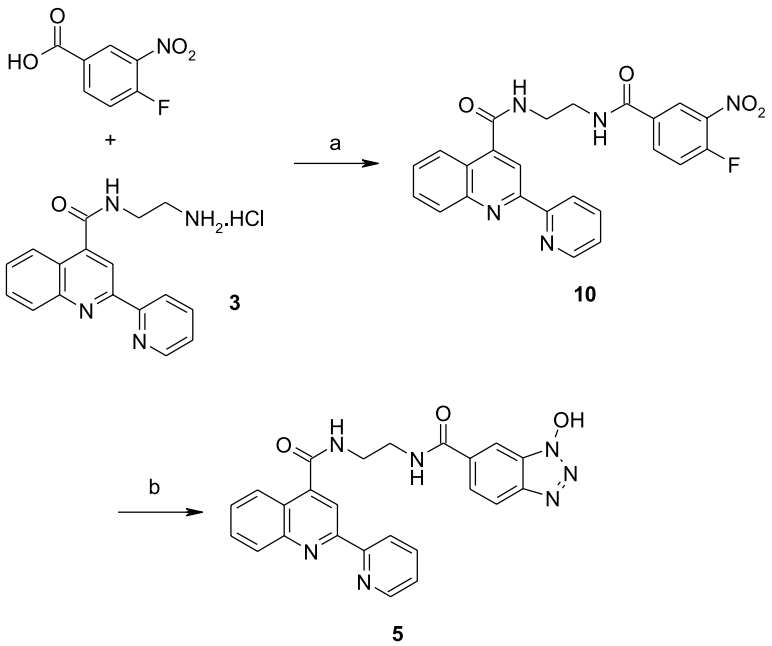
Scheme 2. (a) 2,3,5,6-Tetrafluoro-4-hydroxy benzoic acid, EDC, HOBt, Et_3N, DMF, 50 °C, 80%; (b) phenylacetic acid, PyBrop, DIPEA, DMF, rt.



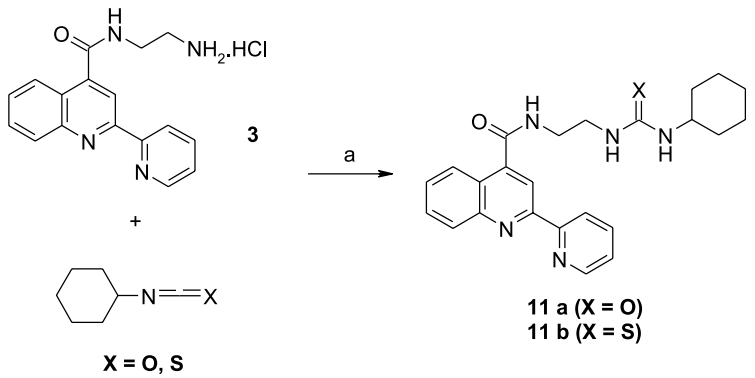
Scheme 3. (a) Phenylacetic acid, PyBrop, DIPEA, DMF, rt; (b) $BnNH_2$, rt, 35%.

The bipyridyl bound tetrafluorophenol analogue **4** was then synthesised[1,5] by coupling amine **3** and 2,3,5,6-tetrafluoro-4-hydroxybenzoic acid (commercially available as a hydrate) in good yield (Scheme 2). Formation of the activated ester was then attempted with phenylacetic acid. Test reactions under different coupling conditions (PyBrop/DIPEA, TBTU/DIPEA, EDC/DIPEA in DMF) were investigated and showed the best result with PyBrop/DIPEA by LC–MS analysis. Unfortunately, although LC–MS showed the formation of the product, it was not possible to isolate it because of its poor stability. This may have been due to the competing formation of acyl pyridinium species in the reaction mixture. The formation of the activated ester and the displacement with benzylamine was then attempted as a one-pot procedure (Scheme 3). After work-up and purification by chromatography, the expected compound was isolated with 35% yield. Whilst this demonstrated that the bipyridyltagged tetrafluorophenol did function as a coupling reagent it was not considered useful as the activated esters could not be isolated and thus the utility of the bipyridyl tag could not be demonstrated.

In the literature, the use of a polymer supported 1-hydroxybenzotriazole derivative has been reported.[6a,6b] We thus decided to synthesise the bipyridyl bound HOBt derivative **5**



Scheme 4. (a) PyBrop, HOBt, DIPEA, DMF, rt, 100%; (b) hydrazine hydrate, EtOH, reflux, 36%.



Scheme 5. (a) Et_3N, DCM, rt, 92% (**11a**), 72% (**11b**).

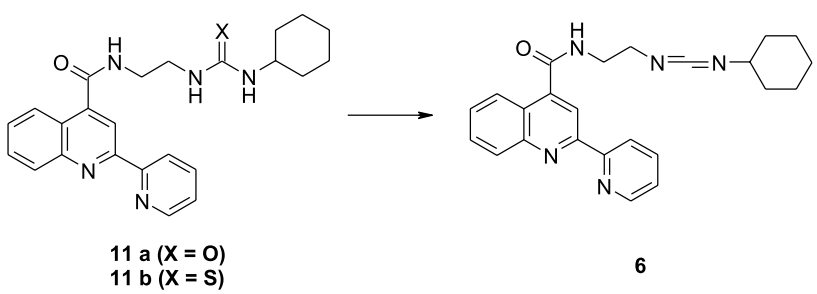
and assess it as a coupling reagent in the hope that it would yield more stable and isolable activated esters. The first step of the synthesis consisted in the coupling between the bipyridyl bound amine **3** and 4-fluoro-3-nitrobenzoic acid (Scheme 4). After purification by chromatography, the expected compound **10** was isolated in a quantitative yield. The second step involved cyclisation using hydrazine hydrate giving triazole **5** which was isolated with 36% yield. Formation of the activated ester was then attempted with Boc-Gly, using EDC/DIPEA in DMF as coupling conditions. Unfortunately, the desired product could not be isolated, probably for the same reason as the tetrafluorophenol derivative.

In light of these results, the synthesis and use of the bipyridyl bound carbodiimide **6** was then considered as this was expected to be much more stable than the activated esters. In order to synthesise this carbodiimide, the urea **11a** was prepared through the reaction between the amine salt **3** and cyclohexylisocyanate in the presence of triethylamine (Scheme 5). The expected compound was isolated as a white solid with an excellent yield. As the carbodiimide could potentially be obtained through the dehydration of the urea or the thiourea, the thiourea **11b** was also prepared through the reaction between the amine salt **3** and cyclohexylisothiocyanate in the presence of triethylamine. The expected compound was isolated as a white solid with 72% yield. Many different methods were attempted for the dehydration of the urea or thiourea (Scheme 6): TsCl under solid–liquid phase-transfer catalytic conditions,[7] TsCl/pyridine, MsCl/Et_3N/DMAP, $SOCl_2/Et_3N$, Ph_3PBr_2/Et_3N,[8] phosgene iminium chloride/Et_3N.[9] None of these methods gave the expected compound.

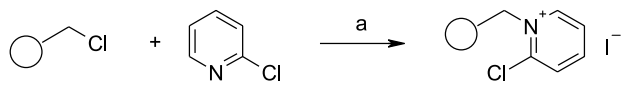
A further method was then attempted with the thiourea **11b**, using Mukaiyama's reagent[10] in solution. An analysis by IR showed the formation of the carbodiimide (stretch at 2121 cm^{-1}), but purification of the product was very difficult (removal of the reaction side-product). In the best conditions, pure carbodiimide was isolated after several stages of column chromatography in 61% yield. Due to these problems of purification, we then employed a polymer-bound Mukaiyama's reagent. This reagent was prepared through the reaction of Merrifield's resin and 2-chloropyridine in the presence of potassium iodide (Scheme 7). Further details relating to the preparation and use of this reagent will be described elsewhere. This reagent was then used for the dehydration of the thiourea **11b**. After filtration and washing of the resin, carbodiimide **6** was obtained in good yield.

Carbodiimide **6** was then tested as a coupling reagent in the reaction between phenylacetic acid and benzylamine. Several test reactions under different conditions were carried out. The use of carbodiimide in combination with DIPEA in DCM solvent gave the best results. After purification by chromatography, the expected compound was isolated with 28% yield. Having demonstrated that **6** could function as a coupling reagent we then carried out some reactions in which the crude reaction mixture was subjected to purification by extraction of the bipyridyl-tagged material onto a Cu-derivatised resin.

During the preliminary experiments of capture of the urea **11a** by the Cu-resin, it appeared that the poor solubility of this compound in DMF or DCM was a significant problem. Therefore, it was decided that a more soluble carbodiimide (and hence urea) should be prepared. It was thought that the introduction of a methyl group on the amide nitrogen would increase the solubility of the compound. A new bipyridyl bound amine, with three carbons as a spacer, could easily be obtained through the coupling between the acid **8** and the

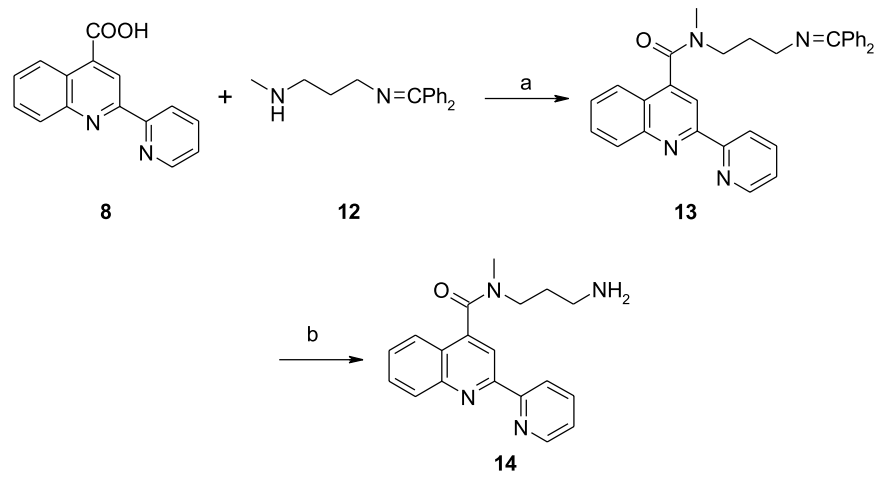


Scheme 6.



Scheme 7. (a) KI, DMF, 60 °C.

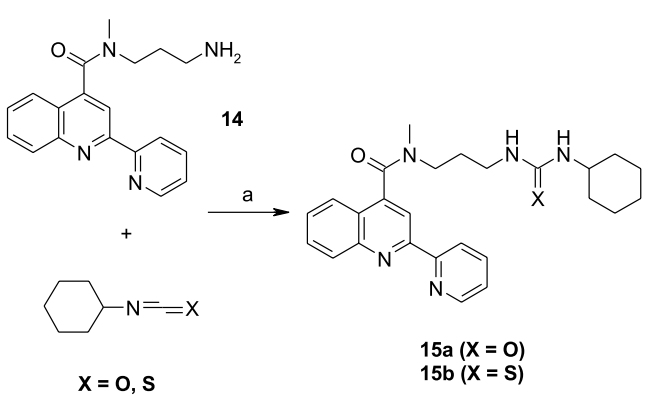
presence of a triethylamine salt impurity. In order to remove this impurity, different work-up conditions were tried: wash with a saturated bicarbonate solution or wash with water. In both cases, a significant loss of mass was observed, and the carbodiimide was partially decomposed.



Scheme 8. (a) EDC, HOBt, DMF, rt, 100%; (b) TFA/H_2O, DCM/THF, rt, 89%.

monoprotected *N*-Me-1,3-propanediamine **12**. For this purpose, the primary amine of the *N*-Me-1,3-propanediamine was protected as a benzophenone imine. The expected compound **12** was isolated with a quantitative yield. The bipyridyl bound amine intermediate **13** was then obtained through the coupling between the acid **8** and the monoprotected *N*-Me-1,3-propanediamine **12** (Scheme 8). The free amine **14** was easily obtained[11] by treatment of the coupled product with aqueous acid.

In order to test our hypothesis regarding solubility, the urea **15a** was then prepared through the reaction between the amine **14** and cyclohexylisocyanate (Scheme 9). The expected compound was isolated as a white solid with an excellent yield and was found to be readily soluble in DMF or DCM solvent. The corresponding carbodiimide was obtained from the thiourea **15b** which was prepared through the reaction between the amine **14** and cyclohexylisothiocyanate. Polymer-bound Mukaiyama's reagent was again used for the thiourea dehydration (Scheme 10). After filtration and wash of the resin, carbodiimide **16** was obtained in good yield but was contaminated by the



Scheme 9. (a) Et_3N, DCM, rt, 94% (**15a**), 75% (**15b**).

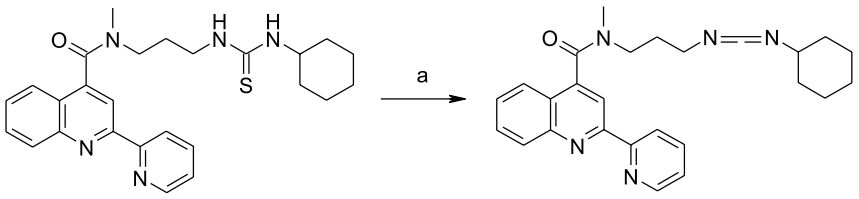
Finally use of a carbonate resin (prepared using Ambersep 900 OH and a solution of sodium carbonate) gave the pure carbodiimide **16** in 26% yield.

This compound was then used as a coupling reagent in a test reaction between phenylacetic acid and benzylamine. A classic work-up was done and after purification by chromatography, the expected coupled product was isolated with 40% yield. The same reaction was accomplished again, and then the Cu-resin was used to extract the side-product of the reaction (see Section 4). LC–MS analysis after 24 h of scavenging showed the coupled product and a small trace of the bipyridyl-tagged urea (<2% by UV in the LC trace). Evaporation of the reaction mixture gave a pink material which, after quick filtration through a plug of silica, to remove residual copper salts, gave the expected compound with 65% yield.

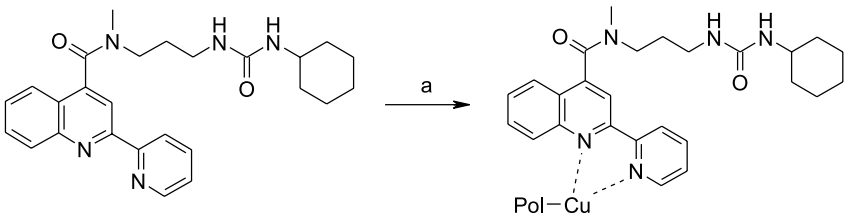
Whilst the carbodiimide could be used as a coupling reagent the difficulty of preparing this reagent and the moderate yield obtained for the coupling reaction led us to consider preparing bypyridyl tagged scavengers instead. We considered using bipyridyl bound amine **14**, which had already been prepared, as an isocyanate scavenger, as the resulting urea **15a** could be removed by extraction with the Cu-resin (Scheme 11). Tests for the capture of the urea **15a** were first accomplished in DCM and in DMF. In order to determine the best conditions for this scavenging a Design of experiments (DOE) approach using the MODDE software from Umetrics was employed.[†]

Experiments were designed which investigated the following parameters: solvent (DCM or DMF), resin quantity, time and amount of water added (Ley and co-workers had noted the requirement for the addition of water in their scavenging process[3]). The extent of scavenging was measured by

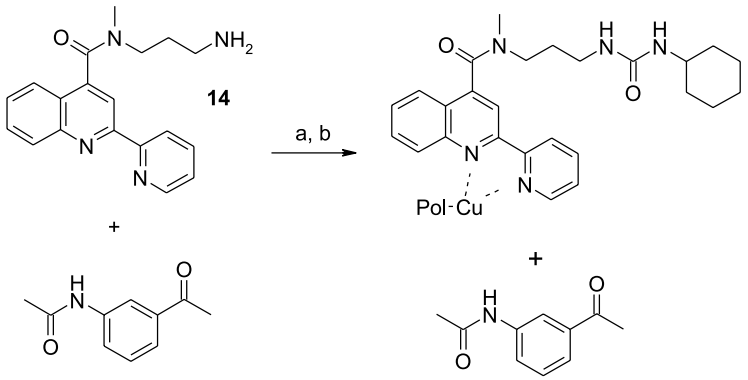
[†] For further information see www.umetrics.com



Scheme 10. (a) PS-Mukaiyama reagent, Et_3N, DMF, 45 °C, 26%.



Scheme 11. (a) DCM or DMF, Cu(II)-resin, 3-acetylacetanilide.



Scheme 12. (a) Cyclohexylisocyanate, DCM, Et_3N; (b) Cu(II)-resin.

LC–MS analysis giving the ratio of residual bipyridyl-tagged urea to an internal standard (3-acteylacetanilide). The use of DMF as a solvent was quickly eliminated due to problems with leaching of Cu from the resin resulting in the formation of blue solutions. A screen of 11 reactions was conducted based on a two-level full factorial design (with 3 centre points) varying the quantity of resin (2–10 g/mmol), amount of water added (10–50 μl) and the time (2–30 h) in DCM solvent. The results from this screen suggested that the main factors were the quantity of resin and the amount of water added. The optimiser function available in the MODDE software then enabled the identification of optimal scavenging conditions. These optimal conditions for scavenging were confirmed to be 10 g/mmol of resin (resin loading 2 mmol/g of Cu^{2+}), 1.8 mL/mmol water in DCM solvent for 24 h after a further two experiments had been conducted.

Once the optimum conditions for the scavenging of urea were established, it was then decided to test this method for isocyanate scavenging. Cyclohexylisocyanate was scavenged using the bipyridyl bound amine **14** in the presence of the internal standard (3-acetylacetanilide), and the thus formed urea was removed from the reaction mixture using the Cu-resin (Scheme 12). After 24 h, LC–MS of the reaction mixture showed that there was less than 5% of the urea remaining. After filtration and passing through a plug of silica (to remove residual copper slats) a white material was obtained, and NMR analysis showed clean 3-acetyl-acetanilide. The generality of this scavenging process is currently under investigation and will be reported in due course.

3. Conclusion

In the course of our investigation we have attempted the preparation of three bipyridyl-tagged reagents and one scavenger. Of the three reagents the carbodiimide derivative proved to be effective as a coupling reagent for amide formation and the removal of the coupling side product from the reaction mixture by complexation onto a Cu-derivatised resin was successfully demonstrated for this case. This said the difficulty of preparing this reagent and its moderate performance in an amide formation detracts from its utility as a coupling reagent of general use. We considered that the

use of such chelation tags for scavenging agents would be more effective. The copper-resin mediated purification process was thoroughly optimised using a DOE approach and the procedure was successfully applied to the use of a bipyridyl-tagged amine as an isocyanate scavenger. These preliminary results clearly demonstrate the potential of using chelation tags such as bipyridine units as a means for removing solution phase reagents and scavengers from reaction mixtures offering an attractive alternative to their resin-bound and fluorous-tagged counter-parts.

4. Experimental

4.1. General methods

All infrared spectra were recorded on a Nicolet 360 FT-IT spectrophotometer. All NMR were recorded on a Bruker AC 400 instrument at 293 K with solutions of compounds dissolved in $CDCl_3$ (unless stated otherwise), referenced to tetramethylsilane (δ 0.00 ppm). Mass spectra were recorded on a Z-spray mass spectrometer. Compounds were dissolved in 1:1 acetonitrile/water and ionised using an electrospray ionisation source and recorded in positive ion mode. Reactions were monitored by TLC using commercially available glass-backed plates (Merck). Column chromatography was carried out on silica gel 60 (40–63 μm, 60A, Fluorochem). All chemicals used in the reactions were reagent grade and used as purchased, except the *N*-Boc-ethylenediamine, and polymer supported Mukaiyama reagent which were synthesised in-house.

4.1.1. Sodium 2-(2-pyridinyl)-4-carboxyquinoline carboxylate 7. Isatin (16 g, 109 mmol) and 2-acetylpyridine (12 g, 99 mmol) were mixed in a beaker. To this mixture was quickly added, while stirring with a spatula, 60 g of a 33% sodium hydroxide solution, which had been previously cooled to 5 °C. The stirring was continued until the contents hardened (temperature 55 °C). 70 mL of water was then added, resulting in a red slurry. This material was then filtered, washed with ethanol (60 mL) and acetone until the filtrate was slightly pink. Crystallisation from hot water and then drying under high vacuum at 40 °C gave the product as a pink powder. Yield: 16.7 g (62%). ^{1}H NMR (400 MHz, CD_3OD) δ: 8.71 (m, 1H); 8.53 (m, 2H); 8.41 (dd, J=8.42, 0.82 Hz, 1H); 8.14 (m, 1H); 7.99 (m, 1H); 7.76 (m, 1H); 7.61 (m, 1H); 7.47 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 175.45, 157.54, 157.35, 150.49, 150.07, 149.73, 138.71, 130.95, 130.46, 128.19, 127.92, 126.32, 125.67, 123.28, 117.41. MS (ES+) m/z: 251 (M−Na+H$^+$). IR: 1573 cm^{-1} (COO$^-$). LC-AccMass: calcd for $C_{15}H_{10}N_2O_2$: 250.074. Found (M+2H−Na)$^+$: 251.08 (+1.1 mDa).

4.1.2. 2-(2-pyridinyl)-4-carboxyquinoline 8. To an aqueous solution of the carboxylate **8** (1 g, 3.68 mmol, dissolved in 20 mL of hot water) was added dropwise a 10% HCl solution to pH=7. Filtration of the solid, washing with acetone and then drying under high vacuum at 40 °C gave the product as a white solid. Yield: 816 mg (89%). ^{1}H NMR (400 MHz, d_6-DMSO) δ: 8.99 (s, 1H); 8.78 (m, 2H); 8.63 (d, J=7.96 Hz, 1H); 8.21 (d, J=7.96 Hz, 1H); 8.05 (ddd, J=7.73, 7.73, 1.74 Hz, 1H); 7.89 (ddd, J=8.37, 6.95, 1.37 Hz, 1H); 7.75 (ddd, J=8.39, 6.98, 1.35 Hz, 1H); 7.57 (ddd, J=7.48, 4.78, 1.12 Hz, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 167.44, 155.08, 154.28, 149.43, 148.15, 137.61, 136.89, 130.24, 129.90, 128.42, 125.53, 125.04, 124.49, 121.02, 119.30. MS (ES+) m/z: 251 (M+H$^+$). IR: 1699 cm^{-1} (COOH). LC-AccMass: calcd for $C_{15}H_{10}N_2O_2$: 250.074. Found (M+H)$^+$: 251.08 (+1.7 mDa).

4.1.3. Amide 9. To a solution of 2-(2-pyridinyl)-4-carboxyquinoline (200 mg, 0.8 mmol) in 4 mL of DMF were successively added *N*-Boc-ethylenediamine (140 μl, 0.88 mmol, 1.1 equiv.), EDC (169 mg, 0.88 mmol, 1.1 equiv.) and HOBt (119 mg, 0.88 mmol, 1.1 equiv.). The reaction mixture was stirred at rt for 5 h 30 min, then diluted with DCM and washed three times with water. Drying over $MgSO_4$, filtration and evaporation gave the crude material. Purification by chromatography on silica gel (2:3 hexane/EtOAc as eluent) gave the product as a white solid. Yield: 312 mg (99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.71 (m, 1H); 8.63 (m, 2H); 8.34 (dd, J=8.51, 0.91 Hz, 1H); 8.18 (d, J=8.25 Hz, 1H); 7.89 (ddd, J=7.73, 7.73, 1.74 Hz, 1H); 7.76 (ddd, J=8.42, 6.95, 1.42 Hz, 1H); 7.60 (ddd, J=8.37, 7.00, 1.28 Hz, 1H); 7.39 (ddd, J=7.50, 4.85, 1.19 Hz, 1H); 7.13 (bs, 1H, NH); 5.11 (bs, 1H, NH); 3.70 (m, 2H, NH−CH_2); 3.47 (m, 2H, NH−CH_2); 1.38 (s, 9H, Boc). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.22, 156.80, 155.56, 155.29, 148.86, 148.41, 142.34, 137.36, 130.12, 130.07, 127.88, 125.50, 124.55, 124.43, 122.07, 116.43, 41.09, 40.82, 29.70, 28.30. MS (ES+) m/z: 393 (M+H$^+$); 337 (M−tBu+H$^+$). IR: 3351, 2981 (NH), 1686, 1641 (C=O) cm^{-1}. LC-AccMass: calcd for $C_{22}H_{24}N_4O_3$: 392.185. Found (M+H)$^+$: 393.19 (+0.2 mDa).

4.1.4. Amine hydrochloride salt 3. To a solution of the Boc-protected compound **9** (1.56 g, 3.97 mmol in 50 mL of DCM) was added 39 mL (20 equiv.) of a 2 M HCl solution in diethyl ether. The reaction mixture was stirred at rt for 1 h, and then filtered. Drying under high vacuum gave the product as a yellow solid. Yield: 1.3 g (99%). ^{1}H NMR (400 MHz, d_6-DMSO) δ: 9.09 (m, 1H); 8.70 (m, 3H); 8.14 (m, 6H); 7.83 (ddd, J=8.21, 7.07, 1.21 Hz, 1H); 7.67 (ddd, J=8.14, 7.14, 1.05 Hz, 1H); 7.59 (ddd, J=7.20, 5.10, 0.66 Hz, 1H); 3.60 (dd, J=11.98, 6.13 Hz, 2H, NH−CH_2); 3.02 (dd, J=11.85, 5.99 Hz, 2H, NH−CH_2). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 166.96, 147.98, 147.34, 142.93, 139.85, 130.67, 129.45, 128.21, 127.23, 125.72, 124.35, 122.31, 119.03, 116.97, 109.70, 38.30, 37.14. MS (ES+) m/z: 293 (M−HCl+H$^+$). IR: 3366, 2966 (NH), 1653 (C=O) cm^{-1}. LC-AccMass: calcd for $C_{17}H_{16}N_4O$: 292.132. Found (M−HCl+H)$^+$: 293.14 (+0.9 mDa).

4.1.5. Urea 11a. To a solution of the bipyridyl bound amine hydrochloride salt **3** (500 mg, 1.52 mmol) in 25 mL of DCM was added cyclohexylisocyanate (215 μl, 1.67 mmol, 1.1 equiv.) and triethylamine (470 μl, 3.35 mmol, 2.2 equiv.). The reaction mixture was stirred at rt for 2 h 30 min. The reaction mixture was then filtered and the solid washed with DCM. Drying under high vacuum gave the product as a white solid. Yield: 583 mg (92%). ^{1}H NMR (400 MHz, d_6-DMSO) δ: 8.94 (t, J=5.12 Hz, 1H); 8.78 (d, J=4.30 Hz, 1H); 8.61 (m, 2H); 8.20 (m, 2H); 8.05 (ddd, J=7.75, 7.75, 1.62 Hz, 1H); 7.87 (ddd, J=8.14, 7.14, 1.05 Hz, 1H); 7.68 (ddd, J=8.00, 7.23, 0.82 Hz, 1H); 7.57 (ddd, J=7.30, 4.92, 0.80 Hz, 1H); 5.90 (m, 2H, NH); 3.40

(m, 2H); 3.27 (m, 2H); 1.74 (m, 2H); 1.61 (m, 2H); 1.51 (m, 1H); 1.28–1.02 (m, 5H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 166.88, 157.51, 155.04, 154.62, 149.31, 147.56, 143.40, 137.51, 130.24, 129.56, 127.62, 125.63, 124.93, 124.23, 121.12, 116.09, 47.77, 45.56, 33.26, 30.65, 25.25, 24.48. MS (ES+) *m/z*: 418 (M+H$^+$). IR: 3241, 2927 (NH), 1627 (C=O) cm^{-1}. LC-AccMass: calcd for $C_{24}H_{27}N_5O_2$: 417.216. Found (M+H)$^+$: 418.22 (+0.4 ppm).

4.1.6. Thiourea 11b. To a solution of the bipyridyl bound amine hydrochloride salt **3** (1 g, 3.04 mmol) in 50 mL of DCM was added cyclohexylisothiocyanate (475 μl, 3.34 mmol, 1.1 equiv.) and triethylamine (940 μl, 6.69 mmol, 2.2 equiv.). The reaction mixture was stirred at rt for 24 h, then washed with water, dried over $MgSO_4$ and evaporated. Purification by trituration in hexane gave the product. Yield: 856 mg (65%). ^{1}H NMR (400 MHz, d_6-DMSO) δ: 9.00 (t, *J*=5.12 Hz, 1H); 8.78 (m, 1H); 8.62 (m, 2H); 8.20 (2×d, *J*=8.28 Hz, 2H); 8.05 (ddd, *J*=7.75, 7.75, 1.67 Hz, 1H); 7.86 (ddd, *J*=8.37, 7.00, 1.33 Hz, 1H); 7.68 (ddd, *J*=8.16, 7.16, 1.07 Hz, 1H); 7.57 (ddd, *J*=7.46, 4.80, 1.10 Hz, 1H); 7.43 (bs, 2H, NH); 3.67 (m, 2H); 3.53 (m, 2H); 1.85 (m, 2H); 1.67–1.53 (m, 3H); 1.31–1.10 (m, 5H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 167.04, 155.01, 154.60, 149.32, 147.58, 143.16, 137.53, 130.27, 129.57, 127.65, 125.63, 124.96, 124.21, 121.11, 116.21, 32.23, 30.66, 25.13, 24.53, 22.02. MS (ES+) *m/z*: 434 (M+H$^+$). IR: 3239, 2929 (NH), 1646 (C=O) cm^{-1}. LC-AccMass: calcd for $C_{24}H_{27}N_5OS$: 433.194. Found (M+H)$^+$: 434.20 (+0.3 ppm).

4.1.7. Carbodiimide 6. To a solution of the thiourea **11b** (100 mg, 0.23 mmol) in 8 mL of DMF were added the polymer-supported Mukaiyama's reagent (892 mg, 10 equiv.) and triethylamine (64 μl, 0.46 mmol, 2 equiv.). The reaction mixture was agitated in a FlexChem oven at 45 °C for 4 h. The resin was then filtered and washed with DMF, MeOH (3 times). After evaporation, the residue was taken in 5 mL of DCM. 750 mg of carbonate resin (previously washed with DCM) was added and the mixture was stirred at rt for 1 h. Filtration and evaporation gave the product. Yield: 19 mg (21%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.73 (m, 1H); 8.67 (m, 2H); 8.34 (dd, *J*=8.46, 0.87 Hz, 1H); 8.21 (m, 1H); 7.90 (ddd, *J*=7.73, 7.73, 1.83 Hz, 1H); 7.78 (ddd, *J*=8.37, 6.91, 1.42 Hz, 1H); 7.62 (ddd, *J*=8.37, 6.95, 1.33 Hz, 1H); 7.39 (ddd, *J*=7.48, 4.78, 1.17 Hz, 1H); 6.61 (bs, 1H, NH); 3.72 (m, 2H); 3.59 (m, 2H); 1.88–1.83 (m, 2H); 1.64–1.59 (m, 2H); 1.48 (m, 1H); 1.31–1.10 (m, 5H). IR: 3267, 2926 (NH), 2116 (N=C=N), 1649 (C=O) cm^{-1}.

4.1.8. Protected amine 12. To a solution of *N*-methyl-1,3-propanediamine (500 mg, 5.67 mmol) in 10 mL of DCM was added benzophenone imine (950 μl, 5.67 mmol, 1 equiv.) under inert atmosphere. The reaction mixture was stirred at rt for 24 h, and then diluted with 20 mL of DCM. Three washes with a 1% bicarbonate solution followed by drying over $MgSO_4$ and evaporation gave the product as a yellow oil. Yield: 1.36 g (95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.59 (m, 2H); 7.39 (m, 6H); 7.16 (m, 2H); 3.43 (t, *J*=6.82 Hz, 2H, $-CH_2-CH_2-CH_2-$); 2.66 (t, *J*=7.04 Hz, 2H, $-CH_2-CH_2-CH_2-$); 2.42 (s, 3H, CH_3); 1.87 (ddd, *J*=13.95, 6.95, 6.95 Hz, 2H, $-CH_2-CH_2-CH_2-$); 1.60 (bs, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 132.42, 130.07, 129.92, 128.51, 128.39, 128.28, 128.08, 127.75, 52.12, 50.41, 36.18, 30.87. MS (ES+) *m/z*: 253 (M+H$^+$). IR: 2930, 2781 (NH), 1619 (C=N) cm^{-1}. LC-AccMass: calcd for $C_{17}H_{20}N_2$: 252.163. Found (M+H)$^+$: 253.17 (−0.6 mDa).

4.1.9. Amide 13. To a solution of 2-(2-pyridinyl)-4-carboxyquinoline **8** (200 mg, 0.8 mmol) in 4 mL of DMF were successively added protected *N*-methyl-1,3-propanediamine **12** (222 mg, 0.88 mmol, 1.1 equiv.), EDC (169 mg, 0.88 mmol, 1.1 equiv.) and HOBt (119 mg, 0.88 mmol, 1.1 equiv.). The reaction mixture was stirred at rt for 20 h, then diluted with DCM and washed three times with water. Drying on Na_2SO_4, filtration and evaporation gave the crude material. Purification by chromatography on silica gel (1:4 hexane/EtOAc as eluent) gave the product. Yield: 318 mg (100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.70–6.80 (m, 19H); 3.72 (t, *J*=7.36 Hz, 2H, $-CH_2-CH_2-CH_2-$); 3.49 (t, *J*=6.77 Hz, 2H, $-CH_2-CH_2-CH_2-$); 3.18 (s, 3H, CH_3); 3.04 (m, 2H, $-CH_2-CH_2-CH_2-$). MS (ES+) *m/z*: 485 (M+H$^+$).

4.1.10. Amine 14. To a solution of amide **13** (2.36 g, 4.88 mmol) in 50 mL of DCM and 50 mL of THF were added 8 mL of water and 8 mL of TFA. The reaction mixture is stirred at rt for 2 h. After evaporation of the solvents, the crude material was taken in water and washed three times with TBME. The aqueous phase was then poured into a saturated bicarbonate solution, then extracted with DCM and dried with K_2CO_3. Evaporation and drying under high vacuum gave the product. Yield: 1.39 g (89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.72 (m, 1H); 8.66 (m, 1H); 8.52 (m, 1H); 8.20 (m, 1H); 7.92–7.72 (m, 3H); 7.59 (m, 1H); 7.38 (m, 1H); 3.79 (t, *J*=7.04 Hz, 1H); 3.25 (m, 2H); 2.97–2.84 (m, 5H); 2.49 (m, 1H); 1.91 (m, 1H); 1.66 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.71, 155.88, 155.65, 149.18, 148.14, 143.60, 137.01, 130.33, 130.15, 127.80, 124.85, 124.61, 124.34, 121.79, 115.86, 39.23, 36.64, 36.48, 31.43. MS (ES+) *m/z*: 321 (M+H$^+$). IR: 3306, 2929 (NH), 1628 (C=O) cm^{-1}. LC-AccMass: calcd for $C_{19}H_{20}N_4O$: 320.164. Found (M+H)$^+$: 321.17 (−0.5 mDa).

4.1.11. Urea 15a. To a solution of the bipyridyl bound amine **14** (200 mg, 0.62 mmol) in 10 mL of DCM was added cyclohexylisocyanate (88 μl, 0.69 mmol, 1.1 equiv.) and triethylamine (95 μl, 0.69 mmol, 1.1 equiv.). The reaction mixture was stirred at rt for 24 h. The reaction mixture was washed with water, dried on $MgSO_4$ and evaporated. Purification by chromatography on silica gel (with MeOH 3% in DCM as eluent) gave the product. Yield: 260 mg (94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.70 (m, 2H); 8.55 (m, 1H); 8.22 (m, 1H); 7.99–7.74 (m, 3H); 7.61 (m, 1H); 7.48–7.38 (m, 1H); 5.62 (bs, 1H, NH); 4.08 (m, 1H); 3.79 (m, 1H); 3.52–3.38 (m, 2H); 3.24 (s, 3H, Me); 1.92 (m, 4H); 1.73–1.49 (m, 3H); 1.42–1.10 (m, 4H). MS (ES+) *m/z*: 445 (M+H$^+$).

4.1.12. Thiourea 15b. To a solution of the bipyridyl bound amine **14** (1.2 g, 3.76 mmol) in 60 mL of DCM was added cyclohexylisothiocyanate (800 μl, 5.64 mmol, 1.5 equiv.). The reaction mixture was stirred at rt for 30 h, then washed with water, dried on $MgSO_4$ and evaporated. Purification by

chromatography on silica gel (85:15 EtOAc/hexane as eluent) gave the product. Yield: 1.3 g (75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.70 (m, 2H); 8.50 (s, 1H); 8.23 (m, 1H); 7.88 (m, 1H); 7.78 (m, 2H); 7.63 (m, 1H); 7.40 (m, 2H); 5.92 (bs, 1H, NH); 3.88 (m, 2H); 3.77 (m, 2H); 2.89 (s, 3H, Me); 2.03 (m, 3H); 1.73–1.49 (m, 3H); 1.42–1.10 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.02, 155.88, 155.45, 149.21, 148.20, 142.84, 137.12, 130.58, 130.32, 128.07, 124.52, 124.34, 123.82, 121.79, 115.97, 44.31, 41.12, 36.85, 32.72, 25.99, 25.33, 24.38. MS (ES+) *m/z*: 462 ($M+H^+$). IR: 3297, 2923 (NH), 1620 (C=O) cm^{-1}. LC-AccMass: calcd for $C_{26}H_{31}N_5OS$: 461.225. Found $(M+H)^+$: 462.23 (−0.4 ppm).

4.1.13. Carbodiimide 16. To a solution of the thiourea **15b** (100 mg, 0.22 mmol) in 8 mL of DMF were added the polymer-supported Mukaiyama's reagent (892 mg, 10 equiv.) and triethylamine (60 μl, 0.43 mmol, 2 equiv.). The reaction mixture was agitated in a FlexChem oven at 45 °C for 4 h. The resin was then filtered and washed with DMF, MeOH (3 times). After evaporation, the residue was taken in 5 mL of DCM. 750 mg of carbonate resin (previously washed with DCM) was added and the mixture was stirred at rt for 1 h. Filtration and evaporation gave the product. Yield: 24 mg (26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.68 (m, 2H); 8.50 (m, 1H); 8.20 (m, 1H); 7.87 (m, 1H); 7.75 (m, 2H); 7.58 (m, 1H); 7.35 (m, 1H); 3.76 (t, J=7.41 Hz, 1H); 3.44 (t, J=6.68 Hz, 1H); 3.25 (m, 2H); 3.01 (m, 1H); 2.95–2.81 (m, 4H); 2.08–0.81 (m, 11H). IR: 2121 (N=C=N), 1628 (C=O) cm^{-1}.

4.1.14. Bipyridyl-tagged tetrafluorophenol 4. To a solution of the bipyridyl bound amine hydrochloride salt **3** (424 mg, 1.29 mmol) in 8 mL of DMF was added triethylamine (400 μl, 2.84 mmol, 2.2 equiv.), 2,3,5,6-tetrafluoro-4-hydroxybenzoic acid (500 mg, 2.19 mmol, 1.7 equiv.), EDC (272 mg, 1.42 mmol, 1.1 equiv.) and HOBt (192 mg, 1.42 mmol, 1.1 equiv.). The reaction mixture was stirred at 50 °C for 24 h. After evaporation, the residue was taken in water (3 mL), triturated, then filtered and washed five times with water (10 mL) to give the product. Yield: 497 mg (80%). ^{1}H NMR (400 MHz, d_6-DMSO) δ: 8.99 (bs, 1H); 8.90 (bs, 1H); 8.76 (d, J=4.48 Hz, 1H); 8.62 (m, 2H); 8.20 (2×d, J=8.37, 8.37 Hz, 2H); 8.05 (ddd, J=7.68, 7.68, 1.33 Hz, 1H); 7.86 (t, J=7.64 Hz, 1H); 7.67 (t, J=7.55 Hz, 1H); 7.56 (dd, J=7.04, 5.31 Hz, 1H); 3.53 (m, 4H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 166.95, 158.07, 155.05, 154.61, 149.29, 147.58, 143.17, 137.52, 130.27, 129.55, 127.60, 125.65, 124.93, 124.21, 121.12, 116.21; 38.75; 38.70. ^{19}F NMR (376 MHz, d_6-DMSO) δ: −144.3; −161.2. MS (ES+) *m/z*: 485 ($M+H^+$). IR: 3273 (NH), 1646 (C=O) cm^{-1}. LC-AccMass: calcd for $C_{24}H_{16}F_4N_4O_3$: 484.116. Found $(M+H)^+$: 485.12 (−2.0 ppm).

4.1.15. Amide 10. To a solution of the bipyridyl bound amine salt **3** (500 mg, 1.52 mmol) in 5 mL of DMF was added DIPEA (795 μl, 4.56 mmol, 3 equiv.). After 5 min, 4-fluoro-3-nitrobenzoic acid (309 mg, 1.67 mmol, 1.1 equiv.), PyBrop (1.06 g, 2.28 mmol, 1.5 equiv.) and HOBt (308 mg, 2.28 mmol, 1.5 equiv.) were added and the reaction mixture was stirred at rt for 66 h. After evaporation of the DMF, the residue was taken in DCM and washed with water. Drying on $MgSO_4$ and evaporation gave the crude material. Purification by chromatography on silica gel (with MeOH 4% in DCM as eluent) gave the product. Yield: 698 mg (100%). ^{1}H NMR (400 MHz, d_6-DMSO) δ: 9.05 (m, 2H, NH); 8.76 (m, 1H); 8.67 (dd, J=7.32, 2.29 Hz, 1H); 8.62 (m, 2H); 8.33 (m, 1H); 8.17 (m, 2H); 8.05 (ddd, J=7.75, 7.75, 1.76 Hz, 1H); 7.85 (ddd, J=8.44, 6.98, 1.40 Hz, 1H); 7.75 (dd, J=11.21, 8.74 Hz, 1H); 7.63 (ddd, J=8.30, 6.93, 1.21 Hz, 1H), 7.56 (ddd, J=7.43, 4.83, 1.12 Hz, 1H); 2.89 (m, 2H, NH–CH_2); 2.55 (m, 2H, NH–CH_2). MS (ES+) *m/z*: 460 ($M+H^+$). IR: 2967 (NH), 1704, 1615 (C=O) cm^{-1}. LC-AccMass: calcd for $C_{24}H_{18}FN_5O_4$: 459.134. Found $(M+H)^+$: 460.14 (+0.9 ppm).

4.1.16. Hydroxybenzotriazole 5. To a solution of amide **10** (700 mg, 1.52 mmol) in 17 mL of EtOH was added hydrazine hydrate (3.315 mL, 106 mmol, 70 equiv.). The mixture was stirred under reflux for 5 h, and then the solvent was removed by rotary evaporation. The residue was taken in 20 mL of cold water and acidified with HCl 1.5 M to pH~4. The product precipitated and was isolated by filtration. Drying under high vacuum at 40 °C gave the product. Yield: 245 mg (36%). ^{1}H NMR (400 MHz, d_6-DMSO) δ: 9.06 (m, 1H); 8.94 (m, 1H); 8.76 (d, J=4.03 Hz, 1H); 8.62 (m, 2H); 8.28 (s, 1H); 8.16 (m, 2H); 8.05 (m, 2H); 7.95 (m, 1H); 7.84 (m, 1H); 7.58 (m, 1H). MS (ES+) *m/z*: 454 ($M+H^+$). IR: 3271 (OH), 2921 (NH), 1639 (C=O) cm^{-1}. LC-AccMass: calcd for $C_{24}H_{19}N_7O_3$: 453.155. Found $(M+H)^+$: 454.16 (−3.1 ppm).

4.1.17. Amide formation using carbidiimide 16 and subsequent chelation purification. To a solution of phenylacetic acid (21 mg, 0.15 mmol) in 420 μl of DCM was added carbodiimide **16** (74 mg, 0.17 mmol, 1.1 equiv.). After agitation at rt for 10 min, benzylamine (17 μl, 0.15 mmol, 1 equiv.) and DIPEA (30 μl, 0.17 mmol, 1.1 equiv.) were added. After 18 h, the reaction mixture was transferred to a fritted syringe containing 1.70 g (10 g/mmol) of Cu-resin with 6.98 mL of DCM and 310 μl of water (1.8 mL/mmol). This mixture was rotated at rt for 24 h. Filtration and evaporation gave a crude material which was taken in DCM and filtered through a plug of silica. This purification gave the clean-coupled product. Yield: 23 mg (65%). ^{1}H NMR (250 MHz, $CDCl_3$) δ: 7.37–7.05 (m, 10H, Ph), 5.58 (bs, 1H, N*H*), 4.40 (d, 2H, CH_2, J=5.83 Hz), 3.55 (s, 2H, CH_2). LC–MS: calcd for $C_{15}H_{15}NO$: 225.29. Found 226.25 $(M+H)^+$.

4.1.18. Copper(II)-containing beads. Amberlite IRC-718 beads (160 mL) were washed three times with methanol, then shaken for 1 h in a 2 M aqueous solution of copper sulfate (50 g, 100 mL).The blue beads were filtered and washed with water (3×100 mL) followed by TBME (3×100 mL). Drying under high vacuum at 60 °C gave 47.02 g of resin (loading 2 mmol/g of Cu^{2+}).

4.1.19. Isocyanate scavenging. To a solution of cyclohexylisocyanate (18 μl, 0.14 mmol) and 3-acetylacetanilide (50 mg, 0.28 mmol, 2 equiv.) in 2.5 mL of DCM was added the bipyridyl bound amine **14** (50 mg, 0.156 mmol, 1.1 equiv.) and triethylamine (20 μl, 0.14 mmol, 1 equiv.). After 6 h, the reaction mixture was transferred to a fritted syringe containing 1.42 g (10 g/mmol) of Cu-resin with

4.2 mL of DCM and 280 μl of water (1.8 mL/mmol). This mixture was rotated at rt for 24 h. Filtration and evaporation gave a crude material which was taken in DCM and filtered through a plug of silica. This purification gave the 3-acetylacetanilide, clean by NMR.

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