Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2759–2763

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A Solid-Phase Approach to Analogues of the Antibiotic Mureidomycin

Andrea Bozzoli, Wieslaw Kazmierski,* Gordon Kennedy, Alessandra Pasquarello and Angelo Pecunioso

GlaxoWellcome SpA, Medicines Research Centre, via Fleming 4, Verona 37135, Italy

Received 10 July 2000; revised 29 September 2000; accepted 29 September 2000

Abstract—A library of 80 analogues of the antibacterial compound mureidomycin was prepared using solid-phase chemistry techniques.

Introduction

The increasing incidence of resistance amongst pathogenic bacteria is becoming a serious health problem across the world.[1] This has led to attempts to improve the activities of existing antibacterial agents in order to extend their coverage to drug resistant bacteria and also to discover new classes of antibacterial agents with mechanisms of action distinct from the classical agents such as the β-lactams, macrolides, aminoglycosides and tetracyclines.[2] The mureidomycins (Fig. 1) and the related pacidamycins, have recently been described to be inhibitors of bacterial translocase, an essential enzyme involved in the synthesis of the peptidoglycan component of the bacterial cell wall.[3] One of the traits of mureidomycin is its unusual *C*- to *N*-peptide sequence which is caused by the reversal of the peptide chain polarity by the urea within the chain. Alignment of mureidomycin and the UDP-MurNAc-pentapeptide substrate of the enzyme along their respective deoxyribose-uridine/aminoribosyl-uridine moieties shows that the peptide chains have the same polarity caused by the urea and the *meso*-diaminopimellic acid units.

Mureidomycin exhibits strong activity both in vitro and in vivo against strains of the difficult pathogen *Pseudomonas aeruginosa* and is equipotent to cefsoludin and ceftazidime in an in vivo model of *P. aeruginosa* infection in the mouse. It is active against isolated translocases from both *Escherichia coli* and *Staphylococcus aureus* although it lacks whole cell activity against these bacteria. These interesting biological properties, combined with mureidomycin's modular structure (Fig. 1) which is potentially amenable to a combinatorial chemistry approach, make it an attractive synthetic target. In this communication, we wish to report our initial synthesis of a library of analogues of mureidomycin in the search for novel antibacterial compounds.

The cornerstone of the synthetic strategy designed to provide a library of analogues of mureidomycin was the building up of the modular structure anchored to a suitable solid-phase support. Overall, the structure of mureidomycin presents several challenges for the development of solid-phase chemistry including: the choice of anchor point which dictates the chemical strategy for building up the molecule; the introduction of variation into the framework; and the availability of suitable building blocks. The solid-phase synthesis of peptides combines robust procedures with the availability of a large number of amino acids in suitably protected form. This consideration, together with the synthetic efficiency which would be gained by anchoring the uridyl–sugar moiety to a resin followed by introduction of variation along the peptide chain, implied that the best strategy would be to link a suitably modified uridyl building block to the resin and grow the peptide chain in such a way as to be able to deprotect and release the compounds in the final step or steps of the synthetic sequence.

The most obvious point for attaching the sugar to a resin would be via an acetal based linker which is suitable for diols and therefore for ribose but not deoxyribose analogues. The known activity of the ribose-containing

*Corresponding author. Present address: GlaxoWellcome Research, NC 27615, USA; e-mail: wmk25860@glaxowellcome.com

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00560-6

uridyl peptide antibiotics tunicamycin and liposidomycin against translocase[3] implied that substitution of the deoxyribose by ribose could be justified in the first instance for the development of the chemistry in the solid phase. The choice of acetal linker and acid labile protecting groups in the residues of the peptide chain would allow cleavage from the resin and overall deprotection in one step under acidic conditions. However, it was felt that the unusual enamide moiety of mureidomycin would present problems at this stage and therefore this grouping was substituted by a simple amide in the first instance. For the initial library, the branch point residue *N*-β-dimethyl-α,β-diamino propionic acid was substituted by commercially available diaminopropionic acid in order to simplify the chemistry.

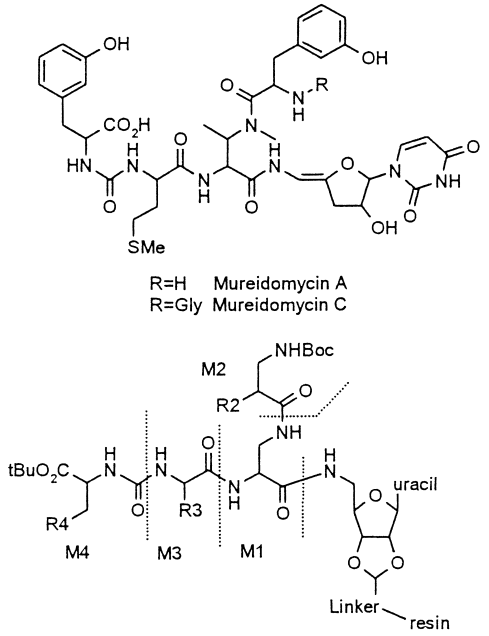


Figure 1. Mureidomycin and the basic scaffold for a library of compounds.

The building blocks were chosen to most closely resemble the residues present in the natural product and, as the absolute configuration of each residue of mureidomycin is unknown, both enantiomers of each building block were included in the design. The enantiomers of diaminopropionic acid M1, methionine M3 and *p*-tyrosine in position M2 and M4 are commercially available, while the two enantiomers of suitably protected *m*-tyrosine M4 were synthesised *ex novo* and *tert*-butyl 2-phenyl malonic acid ester, M4, was prepared as a racemate. The monomers used are shown in Figure 2.

The array of compounds was built up by splitting the loaded resin at each addition of a residue in such a way as to couple each batch of resin produced with the individual monomer residues for that stage. The library was planned to provide a total of 80 (2(M1)×4(M2)×2(M3)×(4+1)(M4)) individuals as discrete compounds.

Results and Discussion

Prior to starting the solid-phase chemistry, the key intermediate acetal protected (5′-*N*-Fmoc-aminoribosyl)-uridine **1** was synthesised in seven steps starting from commercially available isopropylidene uridine (Scheme 1).[4] Conversion of the 5′-hydroxyl function into the azido group occurred smoothly, however, the attempted reduction of the azido group with PPh_3 yielded exclusively the product of Michael attack of the free amine on the uridine ring. In contrast, the amine was successfully obtained by catalytic hydrogenation of the azide in the presence of acetic acid. After Fmoc protection and removal of the dimethylacetonide, the sugar

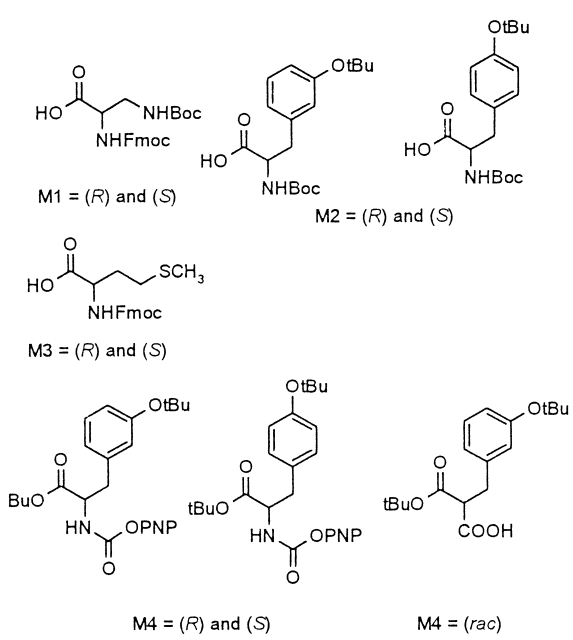
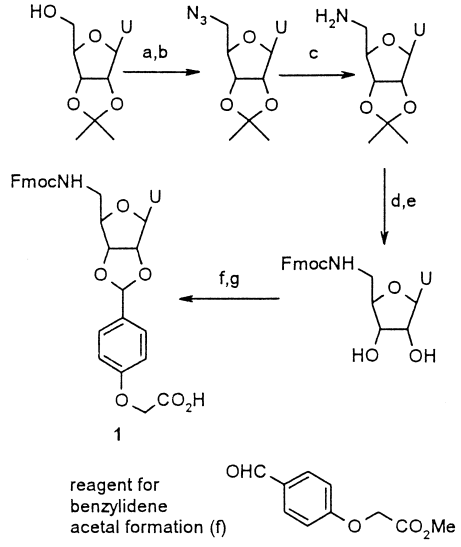


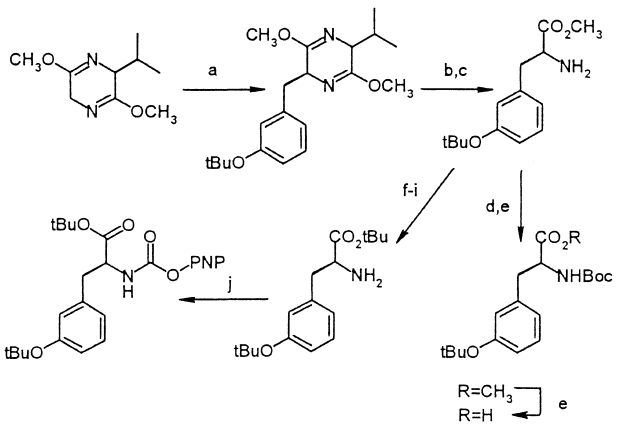
Figure 2. Monomers used in the preparation of the library.



Scheme 1. Reagents: (a) MsCl/DMAP; yield = 80% (15 g scale); (b) NaN_3/DMF/70 °C; yield = 88%; (c) 1 atm H_2/10% Pd/C/MeOH/AcOH; (d) FmocONSu/10% (aq) Na_2CO_3/dioxane; yield = 64% (two steps); (e) TFA/H_2O 95/5; yield = 95%; (f) *p*TsOH/$(EtO)_3CH$/DMF, rt; yield = 89%; (g) Na_2CO_3(aq) 3%, dioxane, rt; yield = 59%.

hydroxyls were protected as the benzylidene acetal through an exchange reaction with the diethylacetal of methyl phenoxy acetic aldehyde pretreated with ethyl orthoformate. The final hydrolysis of the methyl ester was carried out with Na_2CO_3.

Both enantiomers of Boc protected *m*-tyrosine required for the termination of the two chains (M2 and M4) were prepared in enantiomerically enriched form according to the Schöllkopf asymmetric amino acid synthesis (Scheme 2).[5] (*R*)(−)-Bis-lactim ether was reacted with 3-*tert*-butoxy-α-bromotoluene to give the alkylated intermediate. The cleavage of the dihydropyrazine ring was accomplished with 0.25 N HCl without affecting the acid labile *tert*-butyl group.



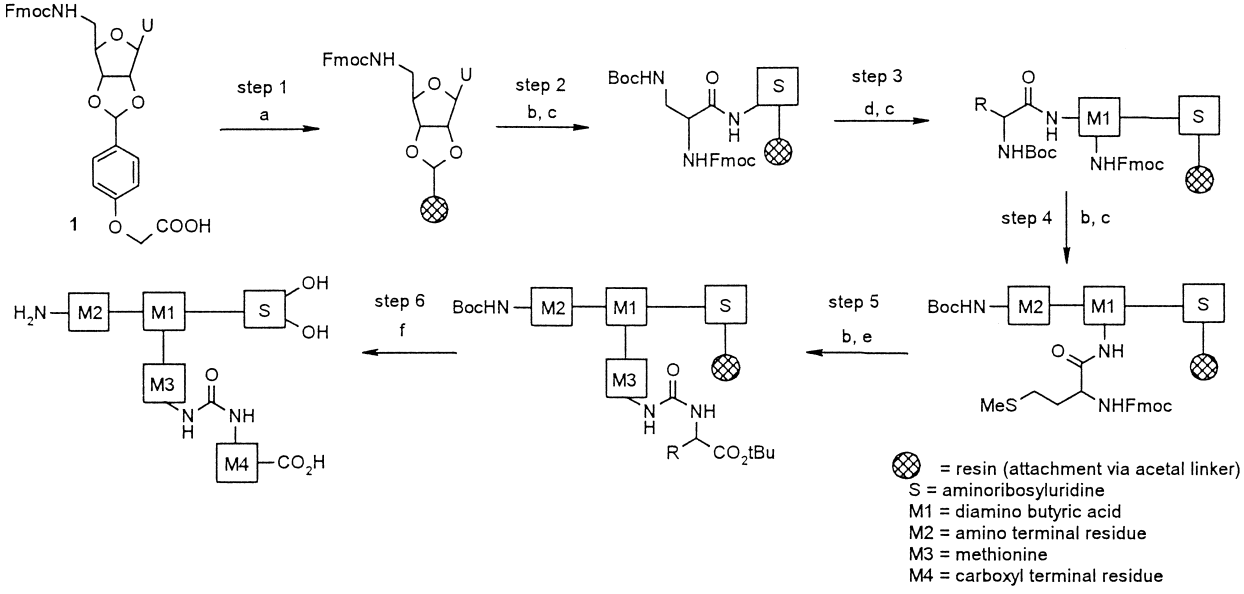
Scheme 2. Reagents: (a) BuLi 1.6 M/−78 °C then 3-(ᵗButoxy)-α-bromotoluene; yield = 70%; (b) HCl 0.25 M; (c) NH_4OH (conc); yield = 79%; (d) Boc_2O/CH_2, Cl_2, rt; yield for b, c, d = 79%; (e) $LiOH{\cdot}H_2O$/EtOH; yield = 94%; (f) *N*-CbzONSu/2 M Na_2CO_3/THF, rt; yield for b, c, f = 61% after chromatography; (g) $LiOH{\cdot}H_2O$/EtOH; yield = 95%; (h) $CCl_3C(=NH)O^tBu/BF_3—Et_2O$; yield = 52%; (i) H_2/10% Pd/C, rt; yield = 93%; (g) *p*-nitrophenylchloroformate/Hünig's base/CH_2Cl_2, rt: yield = 56%; this sequence was repeated with the other enantiomer of the chiral auxiliary to obtain the antipodal *m*-tyrosine.

The crude mixture of (*S*)-*O*-*tert*-Bu-*m*-tyrosine methyl ester and (*R*)-valine methyl ester obtained was treated with di-*tert*-butylpyrocarbonate to give a mixture of (*S*)-*N*-Boc-*O*-*tert*-Bu–*m*-tyrosine methyl ester and (*R*)-*N*-Boc-valine methyl ester which were separated by column chromatography. The enantiomeric excess of the product was determined to be >92% by HPLC analysis after purification and confirmed by NMR analysis of the (+)-methyl mandelate ester.

The tyrosine building blocks used in position M4 were prepared using a less direct route which involved substituting the methyl ester for the acid labile *tert*-butyl ester. Thus (*S*)-*O*-*tert*-Bu–*m*-tyrosine methyl ester (as a crude mixture with (*R*)-valine methyl ester) was treated with *N*-Cbz-OnSu and the resulting mixture chromatographed to remove the valine contaminant. The methyl ester was cleaved and replaced with the *tert*-butyl group[6] and the Cbz group removed by hydrogenolysis. The *p*-nitrophenylcarbamate was formed by reaction of the amine with *p*-nitrophenylchloroformate.[7]

The solid-phase chemistry was rehearsed by synthesising one of the planned library members which was then used as a reference compound during the preparation of the actual library. Thus, the uridine intermediate **1** was linked to an aminomethyl polystyrene (manufacturer-determined loading of 0.9 mmol g^{-1}) through the carboxylate group of the acetal linker (Scheme 3).[8]

The best compromise in using the minimum amount of precious scaffold and having maximum incorporation into the resin was found with 1.1 equiv of scaffold and PyBop as coupling reagent in the presence of Hünig's



Scheme 3. Reagents: see Refs 8 and 11 (a) scaffold-linker (1.1 equiv), PyBop (1.1 equiv), Hünig's base (1.1 equiv), DMF, 18 h, rt; (b) Fmoc-deprotection: 20% piperidine in DMF, rt, 1 h; (c) coupling reaction: PyBop (5 equiv), Hünig's base (5 equiv), M (1–4) (5 equiv), DMF, rt, 18 h; (d) *N*-Boc-deprotection TMSCl/PhOH 1/1 20 M, rt, 4 h; (e) urea in solid phase: *p*-NO_2-carbamate species (5 equiv), Hünig's base (10 equiv), CH_2Cl_2, rt, 18 h; (f) cleavage from the resin: TFA/H_2O 95/5, 2 h, rt.

base. The best loading obtained was 0.534 mmol g^{-1} (equivalent to 92% yield). Unreacted amino groups were capped with 10% acetic anhydride in NMP.

α-Fmoc-β-Boc-D-Diaminopropionic acid (D-M1) was coupled to the resin-linked 5′-aminoribose under standard peptide coupling conditions after Fmoc deprotection and an aliquot of the product cleaved from the resin by treatment with TFA (step 2). The structure of the compound was confirmed by NMR and LC–MS and the yield of this step estimated as 75% by the Fmoc method.[9] Interestingly, no formation of the undesired Michael addition product was detected upon release of the Fmoc group from the bound ribose unit. Selective removal of the Boc-protecting group in the presence of the acetal was rehearsed in solution and the resulting procedure (TMS-Cl/phenol 20 M) was then applied to the reaction in the solid phase.[10] In order to verify that these cleavage conditions were general for the resin bound case, the Boc group was removed, the free amine capped with acetic anhydride in NMP and the *N*-acetyl derivative confirmed after cleavage from the resin. The coupling with D-phenylalanine as a terminal residue in the M2 position was confirmed by HPLC, MS and NMR analysis on a small aliquot cleaved from the support (step 3). After removal of the Fmoc group from M1, the D-methionine residue was coupled to the growing chain and a small quantity of compound cleaved from the resin for analysis (step 4). The overall yield up to this step was estimated to be 55–62% by the Fmoc method.

A number of attempts were made to carry out the formation of the *p*-nitrophenylcarbamate on the resin; however, after removal of Fmoc group, the free amine of the methionine residue reacted with *p*-nitrophenylchloroformate to form a cyclic product which did not react further. The reaction of an excess of *p*-nitrophenylcarbamate of D-phenylalanine *t*-butyl ester (M4) with the resin bound amine in the presence of excess base was more successful and gave the desired product in ca. 50% yield after cleavage from the support (step 5).

The library was prepared with both enantiomers of the available building blocks where possible as shown in Figure 2.[11] The yields of the steps during the synthesis were calculated using the Fmoc reading where appropriate and the Kaiser test[12] was used to check for unreacted bound amine in all steps. The quality of each intermediate in the synthesis was checked by LC–MS analysis of the cleaved compound which showed that up to the formation of the urea the chemistry proceeded without difficulties. All of the compounds in the final library were confirmed by LC–MS although the purities were variable (15–46% a/a). The presence of a peak in the mass spectrum with *m*/*z* 624, corresponding to the compound obtained from step 4, showed that the formation of the urea did not go to completion and that this step is not optimised. A selection of the final compounds are collected in Table 1.

Stock solutions in 500 μL DMSO of 2 mg of each of the final compounds were prepared and, assuming an average purity of 35% for the whole library, diluted with water to give two solutions in which the final concentration of the desired compound was estimated to be 30 and 60 μg mL^{-1}. These solutions were tested for inhibition of growth of *S. aureus* 853, *E. coli* 1852, *Saccharomyces cerevisiae* NCY 81, *P. aeruginosa* and *P. aeruginosa* 2033, however, no activity was detected for any of the products in the library.

Table 1. Analyses of the 10 best final compounds

M1	M2	M3	M4	a/a %[a]
(L)-DAPA	(*R*)-mTyr	(L)-met	(*S*)-mTyr	46
(L)-DAPA	(L)-Tyr	(L)-met	(D)-Tyr	43
(D)-DAPA	(L)-Tyr	(D)-met	(D)-Tyr	42
(L)-DAPA	(*R*)-mTyr	(D)-met	(*S*)-mTyr	40
(L)-DAPA	(L)-Tyr	(L)-met	(*S*)-mTyr	38
(L)-DAPA	(*R*)-mTyr	(D)-met	(L)-Tyr	36
(D)-DAPA	(L)-Tyr	(L)-met	(*S*)-mTyr	36
(L)-DAPA	(L)-Tyr	(D)-met	(*R*)-mTyr	36
(L)-DAPA	(L)-Tyr	(D)-met	(*S*)-mTyr	35
(D)-DAPA	(L)-Tyr	(D)-met	(L)-Tyr	35

[a]Values measured as a/a by HPLC/MS: DAPA = diaminopropionic acid; mTyr = *meta*-tyrosine; Tyr = tyrosine; met = methionine.

While it could be argued that the analogues prepared do not sufficiently resemble the parent compound in their overall structural features, the main thrust of this work was to develop a flexible solid-phase approach to this type of complex molecule. A number of publications containing information relevant to understanding the mechanism of action and structure–activity relationships of the mureidomycins[13] and the related pacidimycins[14] appeared after completion of this work.

It is apparent that the enamide group is not essential for activity against translocase and that its replacement by an amide makes the target analogues synthetically more accessible but does not reduce affinity for the enzyme. The importance of the absolute stereochemistries of the various amino acid residues present in the structure of pacidamycin has been noted by Lee[14] and this issue was addressed in the current work by including both (*R*)- and (*S*)-enantiomers of the monomers in the library described.

The *N*-methyl diaminopimellic acid residue has also been found to be essential for biological activity in the pacidamycin series, and it seems likely that the substitution of this residue by a simpler diamino acid is an important factor in the lack of biological activity observed here. *N*-Methylation is known to affect the *cis*/*trans* geometry of an amide bond and it seems likely that the use of a simple amide in these analogues puts the M2 side chain in a different spatial orientation with respect to that in the natural product. Furthermore, the ribose sugar may not be an optimal substitution for the deoxyribose sugar, however, this may be less important considering the activity observed in the ribose containing tunicamycins and liposidomycins.

Conclusions

Notwithstanding the shortcomings of the compounds made during this work, the original synthetic scheme for

the solid-phase synthesis of analogues of uridyl-peptide antibiotics similar to mureidomycin was successfully validated by the production of an 80-membered library in which all final compounds were confirmed by LC–MS analysis. The biological results obtained may be attributable either to the structural approximations that were introduced to simplify the synthesis in solid phase or a lack of penetration into the bacterial cell. In the light of recently published data, a further iteration of this chemistry incorporating the crucial diaminobutyric acid residue and addressing the ribose/deoxyribose issue should furnish closer analogues of the mureidomycins.

Acknowledgements

We wish to thank our colleagues of the Combinatorial Technology, Lead Generation, Mass Spectrometry and NMR Spectroscopy Laboratories for the support given to this work.

References and Notes

1. Roth, V. R.; Jarvis, W. R. *Emerging Therapeutic Targets* **1999**, *3*, 73. Niccolai, D.; Tarsi, L.; Thomas, R. J. *Chem. Commun*. **1997**, 2333.
2. Setti, E. L.; Quattrocchio, L.; Micetich, R. G. *Drugs Future* **1997**, *22*, 271.
3. Isono, F.; Inukai, M. *Antimicrob. Agents Chemother*. **1991**, *35*, 234. Isono, F.; Inukai, M.; Takahashi, T.; Haneishi, T.; Kinoshita, T.; Kuwano, H. *J. Antibiot*. **1989**, *42*, 667. Brandish, P. E.; Kimura, K.-I.; Inukai, M.; Southgate, R.; Lonsdale, J. T.; Bugg, T. D. H. *Antimicrob. Agents Chemother*. **1996**, *40*, 1640. Inukai, M.; Isono, F.; Takatsuki, A. *Antimicrob. Agents and Chemother*. **1993**, *37*, 980. Isono, F.; Katayama, M.; Inukai, M.; Haneishi, T.; Kinoshita, T.; Kuwano, H. *J. Antibiot*. **1989**, *62*, 674. Brandish, P. E.; Burnham, M. K.; Lonsdale, J. T.; Southgate, R.; Inukai, M. *J. Biol. Chem*. **1996**, *271,* 7609.
4. Pedroso, E. *Tetrahedron Lett*. **1993**, *34*, 2195.
5. Schöllkopf, U. *Angew. Chem., Int. Ed. Engl*. **1981**, *20*, 798; Williams, R. M. *Synthesis of Optically Active α-Amino Acids*; Pergamon Press: Oxford, 1989.
6. Armstrong, A.; Brackenridge, I.; Jackson, R. F. W.; Kirk, J. M. *Tetrahedron Lett*. **1988**, *29*, 2483.
7. Raju, B.; Kassir, J. M.; Kogan, P. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 3043.
8. *Example procedure for anchoring the uridine scaffold to solid phase*: PS-aminomethyl resin (60 mg, 0.9 mmol g^{-1}) was swollen with dry DMF (500 μL) then reacted with uridine scaffold (6.2 mg, 0.0099 mmol, 1.1 equiv), PyBop (5.15 mg. 0.0099 mmol, 1.1 equiv) and DIPEA (1.7 μL, 0.0099 mmol, 1.1 equiv). The suspension was mixed with a rotator at room temperature for 15 h, the resin filtered and washed with DMF, CH_2Cl_2 (three times), MeOH (three times) and again CH_2Cl_2 (three times). Unreacted amino groups on the resin were capped by treatment with a solution of acetic anhydride 10% in 1-methyl-2-pyrrolidinone (1 mL). The suspension was mixed with a rotator at room temperature for 3 h, the resin filtered and washed with DMF, CH_2Cl_2 (three times), MeOH (three times) and again CH_2Cl_2 (three times). Completeness of the reaction was checked by the Kaiser test. The loading (42%) was determined by the Fmoc number reading.
9. Chang, C. D. *Int. J. Pept. Protein Res*. **1980**, *15*, 59.
10. Merrifield, R. B. *J. Org. Chem*. **1993**, *58*, 5167.
11. *Preparation of the library*: From the results obtained in the rehearsal stage, it was possible to estimate the amount of resin bound **1** required for the synthesis of *at least* 2.5 mg of final compound of average MW of ca. 830 as at least 2×1600 mg. The synthesis of the library was carried out in syringes and after each coupling with the monomers, the resin was split into a number of aliquots equal to the number of monomers of the next step. The uridine intermediate **1** was anchored to the aminopolystyrene resin (see note 8) with a yield of 92% (0.534 mmol g^{-1}) for one batch and 81% (0.475 mmol g^{-1}) for the other. Resin bound **1** (2.3 g and 1.9 g) were coupled with L-M1 and D-M1, respectively after Fmoc deprotection. The reactions were compared with the reference compound prepared during rehearsal (yield = 80–85%; LC analysis 100% a/a). Both batches were split into four equal parts and the Boc group removed for coupling with M2 (yield = 80–85%; LC analysis 100% a/a). In this case, one product was cleaved for comparison to the reference product. Each of the eight batches were divided into two parts prior to Fmoc deprotection and subsequent coupling with L- and D-M3 (yield = 85%; LC analysis 100% a/a). For the last step, each of the 16 batches was split into five aliquots for coupling with 5 equivalents of *p*-nitrophenylcarbamate of M4 in the presence of 10 equivalents of Hünig's base after Fmoc deprotection (yield = ca. 50%). Each syringe contained ca. 35 mg of resin and gave 6–7 mg of product after cleavage with aqueous TFA. LC-MS analysis of the final products confirmed the presence of all desired products with purities in the range 15–45% a/a. LC analysis was carried out using a Supelcosil ABZ+ Plus fitted with a diode array detector and MS output: eluents: A = water + 0.01% acetic acid; B = acetonitrile; flow rate 0.8 mL min^{-1}; gradient defined as: 90% A, 10% B for 2 min; gradient to 40% A, 60% B over 6 min; gradient to 20% A, 80% B over 2 min; isocratic elution 20% A, 80% B for 1 min.
12. Kaiser, E. *Anal. Biochem*. **1970**, *34*, 595.
13. Bugg, T. D. H.; Gentle, C. A. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1279. Bugg, T. D. H.; Gentle, C. A.; Harrison, S. A.; Masatoshi, I. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1285.
14. Lee, V. J.; Hecker, S. J. *Med. Res. Rev*. **1999**, *19*, 521.