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Naked-Eye Bead Property Estimation Using a Red Safety-Catch Linker

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The attachment of linker molecules to polymer beads used as insoluble supports for organic synthesis is a frequent requirement. Defined immobilization of these linker molecules before loading selected building blocks is crucial for subsequent transformations. Therefore, the control of the linker attachment is a central task. Because the molecular bodies of linkers are not incorporated in the final molecules, they can often be replaced or modified without affecting the structure of the products that are finally released. Consequently, it seems straightforward to look for coloured substitutes to established linker molecules. By using coloured linkers, visual inspection of the beads enables fast property estimation after attachment and monitoring of losses during synthesis. This very simple estimation does not have a validated loading determination, but is a useful element of straightforward and non-destructive reaction control that has general applicability. Here we present a red azo dye as an alternative to the Kenner safety-catch linker.

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Introduction

On-bead reaction monitoring suffers from the notorious limitations associated with the analysis of insoluble polymer-bound intermediates. Many methods suited to gain the necessary information developed so far are either destructive, time consuming, or require expensive analytical hardware. On-resin NMR,[1,2] IR,[3–5] MS,[6,7] and electrochemical impedance spectroscopy (EIS)[8] experiments are valuable tools, but tend to be elaborate, especially when quantitative information is sought. On the other hand, colorimetric techniques often offer fast, cheap, and simple access to bead property estimation. Thus, colorimetric approaches can represent practical tools to qualitatively or quantitatively monitor solid-phase reactions.[9–11] If reactions are performed in robotic or semi-automated systems in parallel fashion, it is a considerable advantage to be able to estimate the completeness of a reaction by simply looking into the reactor block or on an array of glass reactors instead of taking samples from each vessel. Therefore, further additions to the existing repertoire of colorimetric on-bead reaction monitoring methodology are in high demand.

Results and Discussion

Given the fundamental importance of linker molecules for the synthetic sequence, it is essential to verify completeness of the initial attachment to the beaded resin. Residual polymer bead functional groups might disturb the ongoing reaction sequence and often necessitate additional time-consuming capping and washing steps. Concurrently, loss in loading level encountered at the very beginning of a synthetic sequence will lower the yield of final products obtained to the same degree as the incompleteness of much more delicate reaction steps down the line in a multi-step process.

Support-bound primary amines present in aminomethylated polystyrene have commonly been detected with ninhydrin[12] and other classical reagents (recently reviewed[9]). The still widely used destructive Kaiser ninhydrin test is suited to indicate the absence of amino groups, and can thus serve to monitor the degree of formation of amide bonds between carboxylic acids and aminomethylated polystyrene resins. Similarly, addition of the pH indicator bromophenol blue allows the course of amide bond formation to be followed by continuous visual examination.[13] The change in colour of the coupling slurry from deep violet to blue and finally to green/yellow indicates the consumption of free amino groups and can serve as a signal that the reaction has proceeded as anticipated. This simple yet elegant standard procedure widely used for reaction control during syntheses using construct **1** (Scheme 1) requires no further bases other than the amino group of the polymer taking part in the bond formation. Unfortunately, novel supports like the recently proposed ULTRA resins, invented by Rademan et al.,[14] contain minor amounts of tertiary amines within their functional backbone, and these cannot be subjected to amide bond formation in a preceding capping step. Therefore, they are likely to obstruct the use of pH-indicating dyes such as bromophenol blue in the monitoring of reactions. As this newly

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developed type of high loading resin will almost certainly gain wide spread use in the near future, novel techniques for visual reaction monitoring are needed.

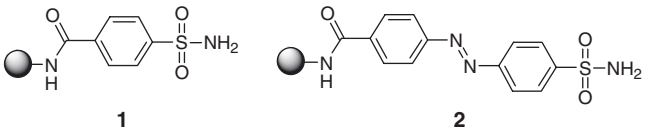
Use of the polymer-bound 4-(4-sulfamoylphenylazo) benzoic acid **2** as a surrogate for the Kenner safety-catch linker 4-sulfamoylbenzoic acid **1** proposed by Ellman et al.[15] (Scheme 1) gives rise to deeply coloured resin beads. Moreover, the depth of the resin colour directly allows for the fast and easy, although approximate estimation of the loading level by naked-eye inspection (Fig. 1).

It is obvious that the incorporation of an azo functionality requires special precautions and that the novel red linker has limitations with respect to possible chemical transformations, especially its stability towards reducing agents. However, it is not the focus of this report to present a novel linker with broad applicability. Instead, we intend to demonstrate the astonishingly simple but useful novel concept of dying the beads by using coloured linker molecules. Other techniques using polymer-bound dyes to monitor pH or reaction processes attach surplus dyes on sites distant from the linker groups, and thus, are only distantly related to this new suggestion. We propose to replace existing linkers with coloured analogues that are able to yield the same products that would have been obtained using the established, colourless linker groups. This point could be proven and is described in the following paragraphs.

Synthetic strategies using, for example, the Vanguard (AdvancedChemTech) series with Teflon reactor blocks (Fig. 2) or the Chemspeed automated synthesis workstations ASW1000 2000 (Chemspeed) with their glass reactor-vessels, could especially profit from use of coloured beads since the user gains the possibility of an external visual bead property control, as well as the possibility of taking photographs for process documentation purposes. A very recent example of a highly original practical application is the development of a self-indicating amine scavenging reagent reported by Bradley and coworkers.[16]

Although the required 4-(4-sulfamoylphenylazo)benzoic acid **5** is known, it is not commercially available. Therefore, a practical synthesis starting from 4-aminobenzoic acid **3** was developed (Scheme 2). Originally, to obtain the corresponding nitroso compound **4**, we envisioned the oxidation of 4-aminobenzoic acid **3** with oxone in either methanol/water[17] or a two-phase system. The rationale for the selection of oxone as an inexpensive oxidizing agent was the need to access the azo linker molecule **5** on a gram scale. The use of oxone is known to lead to various side reactions, such as the formation of polymerization products, and the separation of the mixtures obtained turned out to be laborious and inefficient. Thus, oxidation was undertaken with hydrogen peroxide and the slightly more expensive sodium tungstate in a two-phase system.[18] This route furnished the desired 4-nitrosobenzoic acid **4** in satisfying quality and amounts.

4-Nitrosobenzoic acid **4** was then coupled with sulfanilamide in boiling acetic acid to give azo linker **5**,[19] the purification of which was undertaken by two different routes. Desired compound **5** could be obtained by extraction with ethyl acetate upon systematic lowering of the reaction pH. In particular, the fractions extracted at pH 6–7 were found to contain the protonated acid, but the low solubility of **5** rendered this approach time-consuming. Alternatively, we isolated the sodium salt of target compound **5** at pH 13 in high purity and yield, and furnished the desired 4-(4-sulfamoylphenylazo)benzoic acid **5** upon subsequent treatment with boiling hydrochloric acid.



1 **2**

Scheme 1. Ellman's Kenner safety-catch linker variant **1** and polymer-bound 4-(4-sulfamoylphenylazo)benzoic acid **2**.

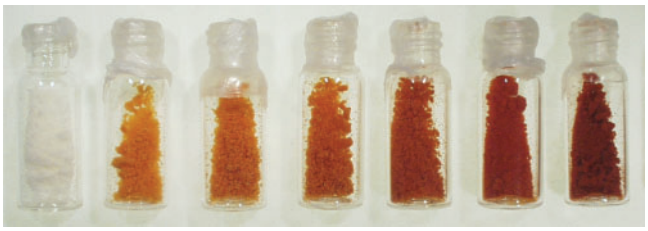


Fig. 1. Prepared test row with increasing loading level. Loading level begins on the left side (0 up to 0.93 mmol g^{-1}). Resins dried from THF.

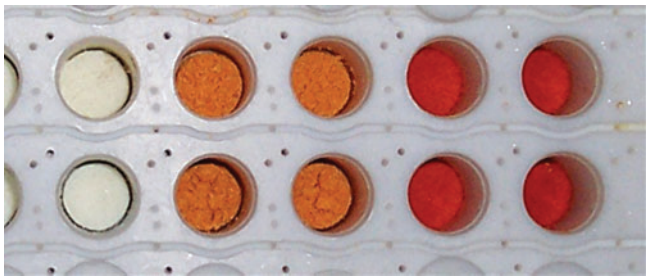
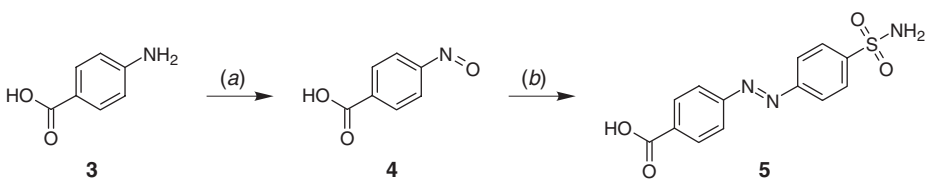


Fig. 2. Resin **2** with different loadings in the Vanguard synthesizer (AdvancedChemTech, Ares Block). Resins were not exhaustively dried.



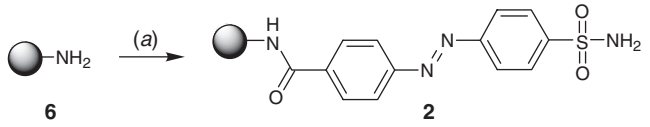
3 **4** **5**

Scheme 2. Synthetic pathway towards 4-(4-sulfamoylphenylazo)benzoic acid **5**: (*a*) H_2O_2, Na_2WO_4, $Bu_4N^+Br^-$, H_3PO_4, 35–40°C, 3 h; (*b*) sulfanilamide, acetic acid, 110–120°C, 4 h.

In the next step, azo dye **5** was coupled onto aminomethylated polystyrene beads **6** under standard conditions[15,20] using *N*,*N*-diisopropylcarbodiimide (DIC), *N*-hydroxybenzotriazole (HOBt), and *N*,*N*-diisopropylethylamine (DIPEA) (Scheme 3).

On-bead synthesis of polymer-bound linker **2** by acylation of aminomethylated polystyrene with 4-nitrosobenzoic acid **4** followed by condensation with sulfanilamide was not envisioned in this project, but could be an interesting topic to be studied in the future.

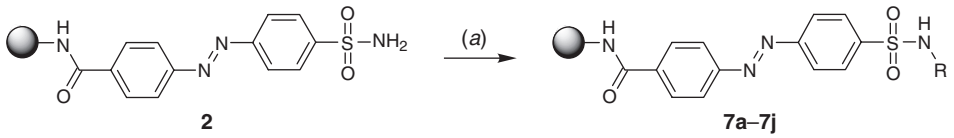
By tuning the amount of azo dye **5** applied in the coupling step from 0.07 to 4.0 equivalents, a row of resin samples with varying loading levels was successfully prepared (Fig. 1), and elemental analysis of the sulfur content indicated that the loading values ranged from 0 to 0.93 mmol g^{-1}. We could easily observe the desired increase in colour, starting with white through to orange and then to a deep red. A test sample with unknown loading level was then prepared, and based upon a visual comparison with the previously composed test row, we predicted a loading level of 0.74 mmol g^{-1}. To verify our prediction, the sample was subjected to elemental analysis, whereby the sulfur content turned out to be 0.71 mmol g^{-1}. It was also determined that the red bead colour is not pH-dependent. Neither addition of 2% trifluoroacetic acid (TFA) nor 2% DIPEA to the swollen polymer resulted in a shift of colour or colour depth. However, the deep red colour could easily be destroyed by treating the beads with tin(II) chloride hydrate in *N*,*N*-dimethylformamide (DMF). Therefore, should additional colour tests further down the reaction sequence appear necessary, a small aliquot of the beads can be decolourized by this widely used reducing reagent. Upon reduction, the azo functionality is destroyed, but the linker bound construct remains attached to the polymer bead. Subsequent colour tests can thereby be performed without being hampered by the strong colour of the beads.



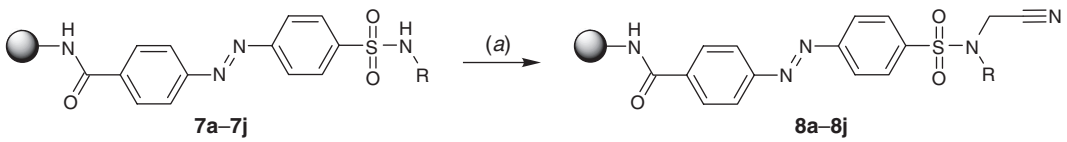
Scheme 3. Coupling of 4-(4-sulfamoylphenylazo)benzoic acid **5** onto aminomethylated polystyrene **6**: (*a*) DIC, HOBt, DIPEA, DMF.

Despite all this, the capability of the new linker to transfer carboxylic acid moieties onto amines still had to be demonstrated. For the preparation of the polymer-bound acylating reagents **7a**–**7j**, ten commercially available carboxylic acids were immobilized onto construct **2** by in situ anhydride formation (Scheme 4).[15] Independently, a natural product derived amine scaffold **9** was obtained by classical solution-phase techniques as recently reported by Lerner et al.[21] After activation of the safety-catch linker with bromoacetonitrile (Scheme 5),[15] the carboxylic acid residues **a**–**j** were successfully transferred onto previously synthesized adenosine template **9** to form the corresponding amides **10a**–**10j** (Scheme 6). Complete conversion to the desired products **10a**–**10j** could be observed by thin-layer chromatography (TLC) after 16 hours. Thus, ten novel adenosine derivatives with putative biological activity[22,23] could easily be made available in good to excellent yields and purities. The novel compounds reported here are intended for ongoing drug design studies pertaining to molecular recognition in ATP-binding proteins and are of importance for understanding enzymatic mechanisms.

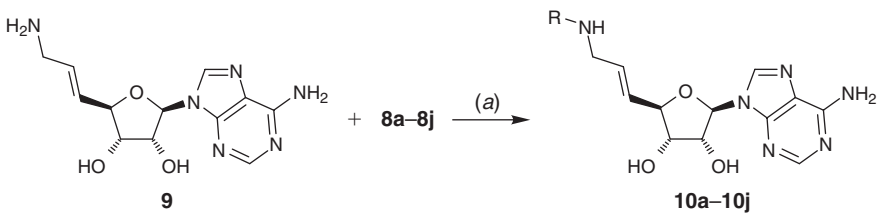
All samples were routinely purified by MPLC to remove particulates and undetectable impurities as recently recommended by Yan et al.[24] Products **10a**–**10j** (Scheme 7) were also characterized by HPLC, ^{1}H NMR spectroscopy, and high



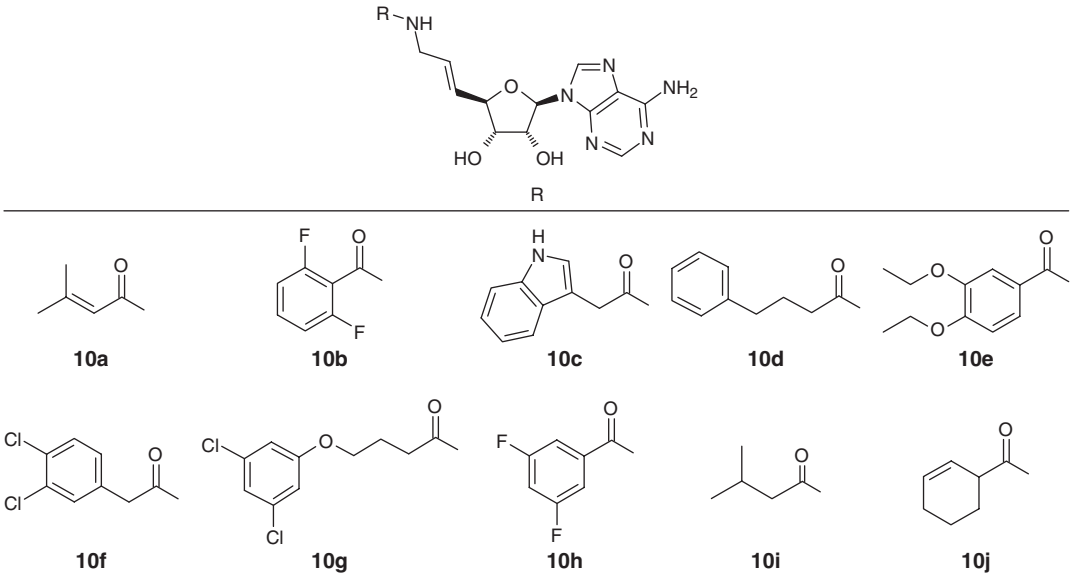
Scheme 4. Coupling of the carboxylic acid building blocks **a**–**j** onto construct **2**: (*a*) DIC, DMAP, DIPEA, THF.



Scheme 5. Activation of the polymer-bound carboxylic acid building blocks **7a**–**7j** with bromoacetonitrile: (*a*) DIPEA, NMP, bromoacetonitrile.



Scheme 6. Transfer of the activated polymer bound building blocks **8a**–**8j** onto amine **9**: (*a*) THF/NMP.



Scheme 7. Structure of compounds **10a**–**10j**.

Table 1. HPLC purities and HRMS (ESI) results for compounds 10a–10j

Compound	Purity [%][A]	Purity [%][B]	Yield [%][C]	HRMS (ESI) (Calc.)	HRMS (ESI) (Found)
10a	98.9	93.5	87	375.1803	375.1781
10b	93.9	95.1	96	433.1440	433.1436
10c	92.1	91.9	96	450.1913	450.1890
10d	97.9	99.7	100	439.2102	439.2094
10e	68.2	86.2	100	485.2130	485.2146
10f	99.2	99.8	99	479.0990	479.1001
10g	92.5	95.6	98	545.1102	545.1083
10h	97.2	92.9	93	433.1460	433.1436
10i	78.5	83.5	95	399.1784[D]	399.1757[D]
10j	97.7	94.5	87	401.1961	401.1937

[A] Relative purity was determined by HPLC using the 100% method, UV detection at 220 nm.
[B] Relative purity was determined by HPLC using the 100% method, UV detection at 254 nm.
[C] Yield was determined through the conversion rate detected at 254 nm.
[D] Calculated and detected mass in this case is $[M + Na]^{+\bullet}$.

resolution mass spectrometry (HRMS (ESI)). The results are shown in Table 1.

Conclusions

The limited possibilities of straightforward non-destructive bead property analysis still hamper the use of polymer-bound reagents. Through insertion of a phenylazo moiety into the Kenner linker modified according to Ellman et al.,[15] a novel red linker was obtained that did not affect the functionality of the safety-catch principle. Thus, we have contributed to currently known techniques for visual bead property estimation for the first step of every polymer related synthesis step, namely attachment of the linker onto the resin. The depth of colour allows for a naked-eye approximation of the resin loading, but if a validated and exact determination was desired, other analytical methods would be necessary. Use of the novel linker for high yielding syntheses of demanding nucleoside derivatives was successfully demonstrated.

Experimental

The structures of all compounds were assigned by NMR spectroscopy. NMR spectra were recorded on a JEOL ECLIPSE+500 spectrometer using tetramethylsilane as internal standard. Test samples prepared in mg quantities were evaluated by HRMS. Purity of the latter compounds was deduced from ^{1}H NMR and LC data. HRMS data were obtained on a Micromass Autospec (ESI, methanol/water 1 : 1 v/v, infusion at 10 μL min^{-1} with polyethylene glycol as reference) instrument. Preparative column chromatography was performed on silica gel 100–200 active, 60 Å, from ICN, or Dowex OH^- (1 × 2–200) using glass columns (4.5 × 15 cm). TLC reaction control was performed on Macherey–Nagel Polygram Sil G/UV$_{254}$ precoated microplates with spots being visualized under UV illumination at 254 nm.

*4-Nitrosobenzoic Acid **4***

This was prepared according to the method of Melnikov et al.[18] 4-Aminobenzoic acid **3** (2.75 g, 0.02 mmol) was dissolved in a three-neck round bottom flask in 1 : 1 dichloromethane/diethyl ether (250 mL) and disodium tungstate dehydrate (0.66 g, 2.0 mmol), phosphoric acid (1.0 mL), tetrabutylammonium bromide (0.20 g, 0.62 mmol), and 30% hydrogen peroxide (20 mL, 0.2 mol) were added. The suspension was then stirred at 35–40°C for 180 min. The organic layer was separated, washed with 0.01 M HCl (100 mL) and water (100 mL), dried over sodium sulfate, and evaporated under vacuum to give **4** (86%). (Found: C 55.5, H 3.5, N 9.3. $C_7H_5NO_3$ requires C 55.6, H 3.3, N 9.3%).

*4-(4-Sulfamoylphenylazo)benzoic Acid **5***

Glacial acetic acid (100 mL) was added to a mixture of **4** (3.02 g, 0.02 mol) and sulfanilamide (3.42 g, 0.02 mol). The resultant mixture

was heated at reflux for 4 h and allowed to cool to room temperature. Small portions of toluene were then added to aid in removing the acetic acid under vacuum. Purification was performed by two different methods.

Method A

The brownish powder was suspended in water (150 mL), concentrated sodium hydroxide solution was added until the solid dissolved, and the solution was washed with ethyl acetate (3×). The resultant red solid that formed in the aqueous layer was then separated by suction filtration, washed with water, and dried under vacuum to afford the sodium salt, mp 385°C (dec.). δ_H (500 MHz; [D$_6$]DMSO) 8.03 (6H, m), 7.82 (2H, d, *J* 8.5), 7.51 (2H, br s).

The sodium salt was subsequently heated at reflux with 1 M HCl for 10 min and the resultant red solid was separated by filtration and dried under vacuum.

Method B

The brownish powder was suspended in water (150 mL), concentrated sodium hydroxide solution was added until the solid dissolved, and the solution was washed with ethyl acetate (3×). The pH of the solution was then adjusted to 6 and the aqueous layer was extracted with ethyl acetate (3×) (because of poor solubility, several fractions had to be collected, separately). The combined organic layers were dried and the solvent was removed under vacuum to afford **5**, mp 321°C (dec.). (Found: C 51.1, H 3.6, N 14.1. $C_{13}H_{11}N_3O_4S$ requires C 51.1, H 3.6, N 13.8%). δ_H (500 MHz; [D$_6$]DMSO) 13.48 (1H, br s), 8.17 (2H, d, *J* 8.5), 8.07 (4H, d, *J* 8.7), 8.02 (2H, d, *J* 8.5), 7.54 (2H, br s).

*General Procedure for the Coupling of 4-(4-Sulfamoylphenylazo) benzoic Acid **5** onto Aminomethylated Polystyrene **6** To Yield Polymer-Bound Linker **2***

N,*N*-Diisopropylcarbodiimide (480 μL, 3.1 mmol) was added to a suspension of HOBt (416 mg, 3.1 mmol) and 4-(4-sulfamoylphenylazo) benzoic acid **5** (935 mg, 3.1 mmol) in THF (20 mL) and the resultant mixture was activated for 4 h (CAUTION: *N*,*N*-diisopropylcarbodiimide may lead to severe allergic reactions, strictly avoid skin contact). Aminomethylated polystyrene **6** (1.0 g, initial loading level 1.40 mmol g^{-1}, 2% cross-linked, purchased from Novabiochem, batch number A25711), pre-swollen in THF (9 mL), was then added to the reaction mixture, which was subsequently shaken for 24 h. The resin was washed with DMF (3×), dichloromethane (3×), and THF (3×), and was then dried under vacuum. Combustion analysis of the sulfur content revealed a loading level of 0.93 mmol g^{-1}.

*General Procedure for the Synthesis of Polymer-Supported Acids **7a**–**7j***

N,*N*-Diisopropylethylamine (150 μL, 0.88 mmol) and 4-dimethylaminopyridine (DMAP) (10 mg, 0.08 mmol) were added to a suspension of dry 4-(4-sulfamoylphenylazo)benzoylaminomethyl polystyrene **2** (500 mg with an initial loading level of 0.93 mmol g^{-1} as determined by elemental analysis) in THF (6 mL), and the resin was allowed to swell at room temperature for 2 h. The appropriate acid **a**–**j** (1.2 mmol, 2.6 equiv.) in a minimum amount of THF was treated with DIC (93 μL, 0.6 mmol) for 4 h before being added to the resin mixture, and the resultant reaction was agitated at room temperature for 24 h. The resin beads were filtered off and washed with DMF (3 × 5 mL), dichloromethane (3 × 5 mL), and THF (3 × 5 mL). After careful drying, the increase in weight was determined. The progress of the reaction could also be followed by IR spectroscopy as acylation of the sulfonamide linker led to a decrease in the intensity of the sulfonamide absorption at 3398 cm^{-1} concurrently with the appearance of a new carbonyl stretch at 1729 cm^{-1}.

*General Procedure for Activation of the Polymer-Bound Carboxylic Acids **7a**–**7j** and **8a**–**8j***

The appropriate resin **7a**–**7j** (approx. 0.2 mmol) in *N*-methylpyrrolidone (NMP) (2 mL) was activated for cleavage by alkylation with bromoacetonitrile (320 μL, 4.5 mmol) (CAUTION: alkylating agent, strictly avoid skin contact) over 12–48 h in the presence of Hünig's base (170 μL, 1.0 mmol). The dark brown slurry was then washed exhaustively with dry dimethyl sulfoxide (5 × 5 mL) and THF (3 × 10 mL).

*2-(3-Aminopropenyl)-5-(6-aminopurin-9-yl)tetrahydrofuran-3,4-diol **9***

This was prepared according to the method of Lerner et al.[21] δ_H (500 MHz; [D$_6$]DMSO + 1 drop D$_2$O) 8.30 (1H, br s), 8.16 (1H, br s), 5.89 (1H, m), 5.79 (2H, m), 4.65 (1H, m), 4.31 (1H, m), 4.10 (1H, m), 3.59 (1H, m), 3.20 (1H, m). δ_C (126 MHz; [D$_6$]DMSO) 156.1, 153.1, 149.7, 140.1, 132.4, 129.2, 119.2, 84.4, 77.8, 74.1, 73.2, 41.9.

*General Procedure for the Synthesis of Compounds **10a**–**10j***

The activated polymer-supported acids **8a**–**8j** (approx. 0.2 mmol) described above were transferred to the amino group of the appropriate amino template **9** (6.0 mg, 0.02 mmol) dissolved in NMP (1 mL) by being shaken at room temperature in THF (4 mL). The reaction mixture was monitored by TLC and terminated upon the quenching of the starting material **9** (12–48 h). Polymer beads and particulates were removed by filtration and the beads were extracted exhaustively with dry THF and methanol. The combined fractions were evaporated to furnish the target compounds **10a**–**10j**.

N-*{(*E*)-3-[(2*R*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl]prop-2-enyl}-3,3-dimethylacrylamide **10a***

Relative purity by HPLC: 98.8% (220 nm), 93.5% (250 nm). (Found (HRESI): $[M+H]^{+\bullet}$ 375.1781. Calc. for $[M+H]^{+\bullet}$ 375.1803). δ_H (500 MHz; [D$_6$]DMSO) 8.28 (1H, br s), 8.14 (1H, br s), 7.85 (1H, t, *J* 5.7), 7.22 (2H, br s), 5.88 (1H, d, *J* 5.1), 5.77 (1H, m), 5.66 (1H, m), 5.47 (1H, d, *J* 5.8), 5.25 (1H, d, *J* 5.9), 4.67 (1H, q, *J* 5.2), 4.31 (1H, t, *J* 6.1), 4.10 (1H, q, *J* 5.3), 3.72 (2H, q, *J* 4.9), 3.32 (1H, m, overlapping H$_2$O), 2.07 (3H, d, *J* 1.1), 1.78 (3H, d, *J* 1.1).

N-*{(*E*)-3-[(2*R*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl]prop-2-enyl}-2,6-difluorobenzamide **10b***

Relative purity by HPLC: 93.9% (220 nm), 95.1% (250 nm). (Found (HRESI): $[M+H]^{+\bullet}$ 433.1436. Calc. for $[M+H]^{+\bullet}$ 433.1440). δ_H (500 MHz; [D$_6$]DMSO) 8.85 (1H, m), 8.26 (1H, br s), 8.14 (1H, br s), 7.50 (1H, m), 7.22 (2H, br s), 7.14 (2H, m), 5.91 (1H, m), 5.87 (1H, m), 5.77 (1H, m), 5.51 (1H, m), 5.29 (1H, m), 4.63 (1H, m), 4.34 (1H, m), 4.11 (1H, m), 3.90 (2H, m).

N-*{(*E*)-3-[(2*R*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl]prop-2-enyl}-2-(1*H*-indol-3-yl)acetamide **10c***

Relative purity by HPLC: 92.1% (220 nm), 91.9% (250 nm). (Found (HRESI): $[M+H]^{+\bullet}$ 450.1890. Calc. for $[M+H]^{+\bullet}$ 450.1913). δ_H (500 MHz; [D$_6$]DMSO) 10.80 (1H, br s), 8.26 (1H, br s), 8.15 (1H, br s), 7.99 (1H, t, *J* 5.7), 7.53 (1H, d, *J* 8.4), 7.32 (1H, d, *J* 8.4), 7.23 (2H, br s), 7.17 (1H, s), 7.04 (1H, m), 6.94 (1H, t, *J* 7.8), 5.88 (1H, d, *J* 5.3), 5.76 (1H, m), 5.69 (1H, m), 5.47 (1H, m), 5.24 (1H, m), 4.63 (1H, m), 4.29 (1H, m), 4.07 (1H, m), 3.71 (2H, m), 3.52 (2H, br s).

N-*{(*E*)-3-[(2*R*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl]prop-2-enyl}-2-(1*H*-indol-3-yl)-4-phenylbutyramide **10d***

Relative purity by HPLC: 97.9% (220 nm), 99.7% (250 nm). (Found (HRESI): $[M+H]^{+\bullet}$ 439.2094. Calc. for $[M+H]^{+\bullet}$ 439.2102). δ_H (500 MHz; [D$_6$]DMSO) 8.28 (1H, br s), 8.14 (1H, br s), 7.92 (1H, t, *J* 5.5), 7.27 (2H, m), 7.22 (2H, be s), 7.16 (3H, m), 5.88 (1H, d, *J* 5.0), 5.78 (1H, m), 5.69 (1H, m), 5.47 (1H, br s), 5.25 (1H, br s), 4.65 (1H, t, *J* 5.0), 4.30 (1H, m), 4.1 (1H, t, *J* 4.9), 3.70 (2H, m), 2.55 (2H, t, *J* 7.7), 2.10 (2H, t, *J* 7.5), 1.79 (2H, m, *J* 7.6).

N-*{(*E*)-3-[(2*R*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl]prop-2-enyl}-2-(1*H*-indol-3-yl)-3,4-diethoxybenzamide* ***10e***

Relative purity by HPLC: 68.2% (220 nm), 86.2% (250 nm). (Found (HRESI): $[M+H]^{+\bullet}$ 485.2146. Calc. for $[M+H]^{+\bullet}$ 485.2130). δ_H (500 MHz; [D_6]DMSO) 8.47 (1H, t, *J* 5.4), 8.29 (1H, br s), 8.08 (1H, br s), 7.44 (2H, m), 7.21 (2H, br s), 6.99 (1H, d, *J* 8.7), 5.89 (1H, d, *J* 5.5), 5.81 (2H, m), 5.48 (1H, m), 5.26 (2H, m), 4.68 (1H, m), 4.32 (1H, m), 4.10 (1H, m), 4.06 (4H, m), 3.90 (2H, m), 3.65 (1H, m), 1.33 (6H, t, *J* 6.7).

N-*{(*E*)-3-[(2*R*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl]prop-2-enyl}-2-(3,4-dichlorophenyl)acetamide* ***10f***

Relative purity by HPLC: 99.2% (220 nm), 99.8% (250 nm). (Found (HRESI): $[M+H]^{+\bullet}$ 479.1001. Calc. for $[M+H]^{+\bullet}$ 479.0990). δ_H (500 MHz; [D_6]DMSO) 8.27 (1H, s), 8.21 (1H, t, *J* 5.7), 8.16 (1H, br s), 7.44 (1H, s), 7.42 (1H, s), 7.29 (1H, t, *J* 8.4), 7.23 (2H, br s), 5.89 (1H, d, *J* 5.0), 5.82 (1H, m), 5.72 (1H, m), 5.49 (1H, d, *J* 5.9), 5.25 (1H, d, *J* 5.5), 4.65 (1H, m), 4.32 (1H, m), 4.11 (1H, m), 3.82 (2H, s), 3.73 (2H, m).

N-*{(*E*)-3-[(2*R*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl]prop-2-enyl}butyramide* ***10g***

Relative purity by HPLC: 92.5% (220 nm), 95.6% (250 nm). (Found (HRESI): $[M+H]^{+\bullet}$ 545.1083. Calc. for $[M+H]^{+\bullet}$ 545.1102). δ_H (500 MHz; [D_6]DMSO) 8.29 (1H, br s), 8.15 (1H, br s), 8.20 (1H, t, *J* 5.6), 7.54 (1H, d, *J* 2.7), 7.33 (1H, dd, *J* 6.2 and 2.7), 7.25 (2H, br s), 7.14 (1H, d, *J* 9.0), 5.89 (1H, d, *J* 5.2), 5.79 (1H, m), 5.69 (1H, m), 5.50 (1H, m), 5.27 (1H, m), 4.66 (1H, m), 4.30 (1H, m), 4.10 (1H, m), 4.05 (2H, t, *J* 6.4), 3.71 (2H, q, *J* 5.2), 2.29 (2H, t, *J* 7.2), 1.95 (2H, m).

N-*{(*E*)-3-[(2*R*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl]prop-2-enyl}-3,5-difluorobenzamide* ***10h***

Relative purity by HPLC: 97.2% (220 nm), 92.9% (250 nm). (Found (HRESI): $[M+H]^{+\bullet}$ 433.1436. Calc. for $[M+H]^{+\bullet}$ 433.1460). δ_H (500 MHz; [D_6]DMSO) 8.81 (1H, m), 8.28 (1H, br s), 8.06 (1H, br s), 7.55 (1H, m), 7.43 (1H, t, *J* 4.4), 7.21 (2H, br s), 5.88 (2H, m), 5.79 (1H, m), 5.46 (1H, m), 5.26 (1H, m), 4.69 (1H, m), 4.33 (1H, m), 4.10 (1H, m), 3.92 (2H, m).

N-*{(*E*)-3-[(2*R*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl]prop-2-enyl}-3-methylbutyramide* ***10i***

Relative purity by HPLC: 78.5% (220 nm), 83.5% (250 nm). (Found (HRESI): $[M+H]^{+\bullet}$ 399.1757. Calc. for $[M+H]^{+\bullet}$ 399.1784). δ_H (500 MHz; [D_6]DMSO) 8.25 (1H, br s), 8.13 (1H, br s), 7.89 (1H, t, *J* 5.5), 7.21 (1H, br s), 5.87 (1H, d, *J* 4.8), 5.71 (2H, m), 4.63 (1H, t, *J* 4.81), 4.29 (1H, t, *J* 5.7), 4.09 (1H, t, *J* 5.0), 3.68 (2H, m), 0.99 (2H, d, *J* 6.4), 0.84 (7H, m).

N-*{(*E*)-3-[(2*R*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl]prop-2-enyl}cyclohex-2-ene-carboxylic Acid Amide* ***10j***

Relative purity by HPLC: 97.7% (220 nm), 95.4% (250 nm). (Found (HRESI): $[M+H]^{+\bullet}$ 401.1937. Calc. for $[M+H]^{+\bullet}$ 401.1961). δ_H (500 MHz; [D_6]DMSO) 8.28 (1H, br s), 8.15 (1H, br s), 7.91 (1H, m), 7.26 (2H, br s), 5.89 (1H, d, *J* 5.0), 5.73 (2H, m), 5.65 (2H, br s), 5.47 (1H, m), 5.24 (1H, m), 4.66 (1H, m), 4.31 (1H, m), 4.11 (1H, m), 3.70 (2H, m), 2.34 (1H, m), 2.09 (1H, m), 2.03 (3H, m), 1.75 (1H, m), 1.51 (1H, m).

Accessory Materials

HPLC profiles of compounds **10a**–**10j** at 250 and 220 nm are available from the author or, until February 2010, the *Australian Journal of Chemistry*.

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